Critical evaluation of methodology commonly used in sample collection, storage and preparation for the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography – mass spectrometry

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ABSTRACT

The main aim of this manuscript is to provide a comprehensive and critical verification of methodology commonly used for sample collection, storage and preparation in studies concerning the analysis of pharmaceuticals and illicit drugs in aqueous environmental samples with the usage of SPE-LC/MS techniques. This manuscript reports the results of investigations into several sample preparation parameters that to the authors' knowledge have not been reported or have received very little attention. This includes: (i) effect of evaporation temperature and (ii) solvent with regards to solid phase extraction (SPE) extracts; (iii) effect of silanising glassware; (iv) recovery of analytes during vacuum filtration through glassfibre filters and (v) pre LC-MS filter membranes. All of these parameters are vital to develop efficient and reliable extraction techniques; an essential factor given that target drug residues are often present in the aqueous environment at ng L^{-1} levels. Presented is also the first comprehensive review of the stability of illicit drugs and pharmaceuticals in wastewater. Among the parameters studied are: time of storage, temperature and pH. Over 60 analytes were targeted including stimulants, opioid and morphine derivatives, benzodiazepines, antidepressants, dissociative anaesthetics, drug precursors, human urine indicators and their metabolites. The lack of stability of analytes in raw wastewater was found to be significant for many compounds. For instance, 34 % of compounds studied reported a stability change > 15 % after only 12 hours in raw wastewater stored at 2 ˚C; a very important finding given that wastewater is typically collected with the use of 24 hr composite samplers. The stability of these compounds is also critical given the recent development of so-called 'sewage forensics' or 'sewage epidemiology' in which concentrations of target drug residues in wastewater are used to back-calculate drug consumption. Without an understanding of stability, under (or over) reporting of consumption estimations may take place.

KEYWORDS: illicit drugs, drugs of abuse, pharmaceuticals, wastewater, surface water, river water, environment, LC-MS/MS, sewage, SPE, multi-residue, silanisation, stability study, sample preparation, sewage forensics, sewage epidemiology

1 INTRODUCTION

Illicit drugs and drugs of abuse are the latest group of emerging environmental contaminants that are receiving a considerable amount of attention [1]. The presence of these compounds in the aqueous environment is important from both a forensic and an environmental perspective. From a forensic

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perspective, the concentration of target drug residues in wastewater influent may be used to back-calculate drug consumption for local communities, thereby offering significant advantages over currently used indirect estimation tools such as population surveys [2]. Furthermore, drug residues present in wastewater can reach surface waters. This is primarily due to insufficient removal at wastewater treatment plants (WWTPs). Therefore, from an environmental perspective, the presence of compounds in the environment has to be investigated as these compounds have been specifically designed to have an impact on humans and could have a negative effect on both humans and wildlife [3].

Consequently, we and several other authors have developed analytical methodologies that study drugs of abuse in the aqueous environment. These methodologies and their analytical aspects have been comprehensively summarised in Table 1. Typically, analytes are analysed through the usage of liquid chromatography (LC) coupled with mass spectrometry (MS). Since most drugs of abuse are present in wastewater influent at ng L^{-1} range, (and lower in effluent and surface waters) it is crucial that an effective pre-concentration procedure is performed to make samples amenable to LC-MS detection limits. This is most commonly carried out through the use of solid phase extraction (SPE), which subsequently involves evaporation of extracts, reconstitution and injection into the LC-MS system. A full understanding of the performance of the above mentioned sample preparation methodology is a critical, although very often overlooked, aspect of the whole analytical process. As can be observed in Tab. 1, certain aspects of sample preparation procedures are in several cases not studied or unreported. Therefore, it might be difficult to draw any constructive conclusions regarding the overall performance of these methods.

There are two major aims of this work; the first is to address some of the important preparation steps that affect recovery of compounds and to the authors' knowledge have not been investigated or received very little attention. The factors investigated include: (i) effect of evaporation temperature and (ii) solvent with regards to SPE extracts; (iii) effect of silanising glassware; (iv) recovery of analytes during vacuum filtration through glassfibre filters and (v) pre LC-MS filter membranes. The second aim is to address perhaps one of the most important factors that to date has not been comprehensively evaluated, the stability of compounds in environmental matrix. Therefore this work presents the results of a detailed stability study of over 60 analytes. Stability is of considerable importance as certain compounds may degrade significantly over a period of only several hours, a factor that must be considered if using a 24 hour composite sampler for collection and if storing samples for any period of time.

2 EXPERIMENTAL

2.1 Chemicals and materials

Analyte names and acronyms, CAS number, molecular formula, log K_{ow}, pK_a and supplier are shown in Table S1. Surrogate/internal standards were all purchased from LGC, with exception of caffeine-d9 (Sigma-Aldrich). All standards and internal standards were of the highest purity available (>97%). Individual stock solutions were purchased or prepared from powdered substance in either acetone or methanol at a concentration of 1 or 0.1 g L^{-1} and stored in the dark at −20 ◦C. Mixed standard solutions were prepared at 10mg L^{-1} in methanol and diluted as necessary to prepare working solutions. Mobile phase solvents and additives were all of LC–MS quality and purchased from Sigma–Aldrich, with the exception of H_2O which was purchased from Fisher. Hydrochloric acid (37%), 5% dimethylchlorosilane (DMDCS) in toluene and ammonium hydroxide (30%) were purchased from Sigma–Aldrich. Ultra pure water was obtained from a water purification system (UHQ-PS, ELGA, UK). Solid phase extraction (SPE) was carried out with Gilson SPE, Aspec XL4 (Anachem, UK). Oasis 60 mg MCX and 60 mg HLB cartridges were purchased from Waters (Waters, UK). SPE samples were eluted into borosilicate glass tubes (12mm x 75mm, Fisher, UK) and evaporated with a TurboVap LV concentration workstation (Caliper, UK). The procedure to deactivate the glassware consisted of rinsing (once) with reagent (5% DMDCS/toluene) for 15 s, toluene (twice) and finally methanol (thrice).

2.2 LC-MS/MS

The aforementioned drug residues and associated metabolites were measured with a fully validated, highly selective and sensitive LC-MS/MS method [4]. Briefly, separation was achieved with the usage of Waters ACQUITY UPLC™ system (Waters, UK) consisting of ACQUITY UPLC™ binary solvent manager and ACQUITY UPLC™ sample manager. Analytes were analysed with an AQUITY UPLC BEH C18 (1.7 µm; 1mm × 150 mm) column, with a mobile phase consisting of mobile phase A (pH 2.9): 79.7%H_2O, 20%MeOH, 0.3%CH_3COOH and mobile phase B (pH 3.3): 99.7%MeOH, 0.3%CH_3COOH at a flow rate of 0.04 mL min^{-1} and a temperature of 30 °C. The gradient programme was as follows: 0min – 100% A, 17 min – 41.3% A, 17.2 min – 0% A, 20.2 min – 0% A, 20.3 min – 100% A, 34.0 min – 100% A. An injection volume of 20 µL was used.

A triple quadrupole mass spectrometer (TQD, Waters, UK), equipped with an electrospray ionisation source, was used for the quantification of target analytes. The analyses were performed in positive mode. The mass spectrometer was operated in selected reaction monitoring (SRM) mode, measuring the fragmentation of the protonated pseudo-molecular ions of each compound. Masslynx 4.1 software (Waters, UK) was used to collect and analyse all data.

2.3 Effect of temperature and elution solvent during evaporation

The optimal temperature for evaporation of the SPE extract was evaluated under a gentle stream of nitrogen (5-10 psi). At this stage in the method development, the use of Oasis® HLB or MCX cartridges was still under investigation, thus the elution solvents used for both cartridges were assessed. Oasis® HLB and MCX elution solvents, methanol (3 mL) and 7 % NH_4OH/MeOH (v/v) (3 mL, pH 11.8) respectively, were spiked with an environmentally realistic amount (50 ng) of each compound (equating to 500 ng/L in the aqueous environment with a sample volume of 100 mL), with exception of creatinine at 400 ng. Samples were evaporated to dryness in silanised vials at temperatures of 20, 30, 40, 50 and 60 °C and removed from the evaporator immediately after the solvent was fully removed. Samples were reconstituted in 0.3 % CH_3COOH, 5 % MeOH/H_2O (v/v) (500 µl).

2.4 Impact of silanisation of glassware

To investigate the interaction of basic molecules with charged silanols on the surface of glassware, the impact of silanising the vials used to hold the SPE extract during the evaporation step was investigated. Oasis® HLB and MCX elution solvent, methanol (3 mL) and 7% NH_4OH/MeOH (3 mL, pH 11.8) respectively, were spiked with 50 ng of each compound, except creatinine at 400 ng. Samples were then evaporated at 40 °C in non-silanised or silanised vials under a stream of nitrogen to dryness, and reconstituted in 0.3 % CH_3COOH, 5 % MeOH/H_2O (v/v) (500 µL).

2.5 SPE method

Following several other optimisation steps including sample volume, wash solvent and elution volume (results not reported), the final SPE procedure was as follows: Initially the Oasis MCX was conditioned with MeOH (2 mL) and equilibrated with 2% HCOOH/H_2O (2mL, pH 2) both at a flow rate of 3 mL min^{-1}. Acidified (pH 1.8 with HCl) river water (500 mL), wastewater influent (100 mL) or effluent (100 mL) were passed through the MCX cartridge at a rate of 6 mL min^{-1}. Immediately following loading, cartridges were washed with 2 % HCOOH/H_2O (2 mL, pH 1.8) at a flow rate of 3 mL min^{-1} and subsequently wrapped in aluminium foil and stored at −20 ˚C no longer than one week before being eluted. Cartridges were washed with 0.6 % HCOOH/MeOH (2 mL, pH 1.8) at a flow rate of 3 mL min^{-1} followed by elution with 7 % NH_4OH/MeOH (3 mL) at a flow rate of 1 mL min^{-1} into silanised vials. Extracts were evaporated to dryness (40 ˚C, N_2, 2–10 psi) and reconstituted with 0.3 % CH_3COOH/5 % MeOH/H_2O (v/v) (500 µL). All samples were filtered through 0.2 µm PTFE filters (Whatman, Puradisc, 13mm) before being transferred to maximum recovery deactivated vials with PTFE septa (Waters, UK).

2.6 Stability study

Key parameters influencing the stability of compounds in wastewater were investigated: pH (2 and 7), temperature (2 and 19 ˚C) and time of storage. Additionally, the influence of suspended solids on stability of analytes in the aqueous phase was evaluated. Stability tests were carried out in duplicate, with all samples stored in the dark in silanised amber glass bottles. All samples were spiked at a concentration of 1000 ng L^{-1} for each compound (in addition to the concentration already present in the wastewater sample, see Table S4). In parallel to the spiked sample, a non-spiked 'control' stability sample was carried out in duplicate alongside each of the spiked samples and processed in exactly the same conditions as those samples which were spiked. Samples were analysed immediately after spiking and then

subsequently at 12, 24 and 72 hour time points. After the extraction of each time point, cartridges were stored at -20 ˚C for a maximum of 7 days before elution. SPE was carried out as described in section 2.5.

Long term storage of compounds on to Oasis MCX sorbent was evaluated. Wastewater (filtered, pH 2) was spiked at a concentration of 1000 ng L^{-1} for each compound and extracted in duplicate as described in section 2.5. After the loading of the sample, cartridges were washed with 2 % (v/v) HCOOH/H_2O (2 mL, pH 1.8), dried, and then stored in an air-tight freezer bag at -20 ˚C. One set of cartridges were eluted immediately, with the remaining cartridges eluted in duplicate after 2, 4 and 6 weeks.

The wastewater influent used in this study was collected as a grab sample (pH of 7.4), after primary screening in a winter month (11/12/10) during relatively dry weather at an urban WWTP (population served 238,000). After the collection of samples, wastewater was transported back to the laboratory on ice and spiked with compounds within five hours.

3 RESULTS AND DISCUSSION

Several sample collection, storage and preparation parameters were investigated. Among them are: (i) effect of evaporation temperature and (ii) solvent with regards to solid phase extraction (SPE) extracts; (iii) effect of silanising glassware; (iv) SPE sorbent; (v) recovery of analytes during vacuum filtration through glass fibre filters and (vi) pre LC-MS filter membranes, and (vii) stability of analytes in environmental matrix at different storage time, temperature and pH. Only the effect of evaporation temperature and solvent with regards to SPE, the effect of silanising glassware, and stability of analytes in environmental matrix at different storage time, temperature and pH are discussed below. Detailed discussion regarding other aspects of this study can be found in the Supplementary Material section.

3.1 Effect of evaporation temperature and solvent

Initially, the optimum temperature for evaporation of SPE extracts was investigated. Attention was paid to the drying of extracts by Clauwaert et al. [5], who found that up to 50 % of free MDA and MDMA was lost on evaporation of compounds in hexane-ethyl acetate at 35 ˚C. Liquid-liquid extraction was used for extraction of these compounds from biological matrices. To improve recoveries, the authors decided to convert the amines to their corresponding hydrochloric salts before drying to ensure non-volatility of the drugs, and prevent the over-drying of extracts for long periods (> 30 min). These modifications resulted in improved recovery and reproducibility. Similarly, Cheung et al. [6] found poor reproducibility of amphetamine and methamphetamine when evaporated to dryness in ethyl acetate. To rectify, samples were evaporated to around 0.2 – 0.4 mL at 40 ˚C before the next analytical step to prevent the over-drying of samples. Jensen et al. [7] examined the evaporation of the well-known herbicide dichlobenil (2,6-dichlorobenzonitrile) and its associated degradation products. During this study, compounds were spiked into acetonitrile and evaporated under nitrogen. The authors found no loss of compounds up to 40 ˚C, while at 50 ˚C a decrease in recoveries of around 25 % was observed for four out of six analytes.

Surprisingly, given the importance of the evaporation step, to the authors' knowledge there are no published manuscripts that discuss the evaporation step with regards to drugs of abuse. In the published material the effect of the evaporation is often taken into account during evaluation of SPE recoveries. However, the effect of evaporation has not been published separate to that of the overall SPE procedure. This results in misleading conclusions potentially being drawn, assigning low recoveries as a consequence of the SPE sorbent when in fact compounds could be lost during the evaporation step. The lack of importance assigned to this variable is shown in manuscripts within this field, with 8 out of the 18 published manuscripts that used evaporation not reporting which temperature was used (see Table 1). In the manuscripts that reported temperature, the majority of procedures used temperatures of 35 or 40 ˚C, with the highest evaporation temperature reported at 45 ˚C [8,9].

In this study, compounds were spiked into two types of elution solvent utilised in Oasis MCX and Oasis HLB methodologies: 7 % NH_4OH/MeOH (v/v) (3 mL, pH 11.8) and methanol (3 mL), respectively. Drying of these extracts was evaluated at 20, 30, 40, 50 and 60 ˚C. Absolute recoveries were calculated against a directly spiked sample diluent standard solution.

When first assessing the impact of temperature in basic methanol (Figure 1a), overall the majority of compounds presented optimal recoveries at 20 ˚C as opposed to the higher evaporation temperatures. A full list of numerical values for the recovery of each compound in basic methanol is supplied in the Supplementary Material section, Table S2. Cocaine and associated metabolites benzoylecgonine, norcocaine, norbenzoylecgonine and cocaethylene showed relatively consistent recoveries over the full spectrum of temperatures, whereas ecgonine methyl ester, anhydroecgonine methyl ester and ecgonidine all showed significant losses over 40 ˚C. For instance, 72 % of ecgonine methyl ester was recovered at 40 ˚C in comparison to 45 % at 50 ˚C. Both amphetamine and methamphetamine were negatively affected by increasing temperature. Evaporation at 40 ˚C for amphetamine and methamphetamine provided respective recoveries of 88 and 89 %, in comparison to recoveries at 50 ˚C of 66 and 68 %. Loss of other amphetamine-like compounds at higher temperatures was not as severe. The vast majority of opioids reported similar recoveries in all temperatures studied, including morphine, codeine, methadone and dihydrocodeine. The benzodiazepines and antidepressants all showed good recoveries between the temperatures 20 – 40 ˚C; with the exception of nitrazepam which decreased by 21 % between the same temperatures. Ketamine and norketamine both showed decreasing recoveries with an increase in temperature.

With regards to temperature in methanol extracts, Figure 1b, the results provided a similar trend to that in basic methanol, although the decrease in recovery with temperature was not as pronounced. A full list of numerical values for the recovery of each compound in methanol is supplied in the Supplementary Material section, Table S3. Cocaine and associated metabolites showed relatively little change in recovery with temperature, including ecgonine methyl ester and ecgonidine which had previously shown lower recoveries. On the other hand, anhydroecgonine methyl ester still showed loss at higher temperature, with a recovery of 73 % at 40 ˚C and 61 % at 50 ˚C. Amphetamine and methamphetamine, in contrast to basic methanol, did not show any pattern with changes in temperature in methanol. The majority of opioids also showed consistent recoveries over the temperature range. Benzodiazepines and antidepressants presented recoveries which were similar with all evaporation temperatures, with the exception of nitrazepam. Ketamine and norketamine, as in basic methanol, showed a decline in recovery with higher temperatures.

In this study, heroin reported low recovery in basic methanol elution solution. Similarly, Bones et al. [10] during SPE method found the Strata XC with basic methanol elution solvent to provide the highest recovery for the majority of their selected compounds, with the exception of a low heroin recovery (26 %). The authors of this paper speculated that this may be due to hydrolysis of heroin to morphine under acidic sample conditions, as morphine achieved an increased recovery of 124 %. The data from this study would in fact suggest that the low recovery could be due to decomposition during the evaporation step. In contrast to Bones and co-workers, in this study the decrease in heroin appeared to reflect deacetylation with an increase in 6-acetylmorphine observed and no apparent increase in morphine (6-acetylmorphine was not monitored by Bones et al. [10] so cannot be compared). Low recoveries of heroin were reported by Rook et al. [11] while developing a method to analyse plasma using an Oasis MCX sorbent with basic methanol elution solvent. The authors of this work rectified this problem and increased the recovery of heroin from 40 to 93 % by lowering the temperature of the eluent to -20 ˚C during SPE elution, shortening the elution time to 10 seconds, and collecting the elution solvent in an acidic buffer before evaporating the solvent. Without this observation, low heroin recovery would have probably been attributed to the SPE performance rather than the evaporation step.

In contrast to heroin, fluoxetine reported low recoveries over the temperature range (25 - 55 %) when evaporated in methanol as opposed to basic methanol. Gros et al. [12] extracted fluoxetine and 28 other multi-class pharmaceuticals using Oasis HLB sorbent with methanol elution solvent. The

authors generally reported low recovery (decoupled from matrix effects) of fluoxetine at two concentrations in WWTP influent (1000 ng L^{-1} = 67 %; 10,000 ng L^{-1} = 108 %), WWTP effluent (100 ng L^{-1} = 74 %; 1000 ng L^{-1} = 60 %), and river water (50 ng L^{-1} = 74 %; 1000 ng L^{-1} = 105 %). Although it cannot be said with any certainty, it may be that low recovery in this method for fluoxetine was due to the effects of the evaporation step. Other published methods for the analysis of fluoxetine used an Oasis MCX with basic methanol elution solvent [13] and an Oasis HLB with 70 % methanol in 2 % acetic acid elution solvent [14], with both papers reporting recoveries greater than 87 %.

Amphetamine showed an approximately 20 % decrease in recovery when evaporated in basic methanol as opposed to methanol at temperatures between 20 – 40 ˚C. Similarly, morphine showed a loss when recovered from methanol. Benzodiazepines presented similar results in both solvents, although a slight increase of around 10 % in all compounds was observed in methanol at the majority of temperatures. Antidepressants, with the exception of venlafaxine, generally showed losses of around 20 % in methanol, although this loss was higher in the case of fluoxetine and its metabolite norfluoxetine.

In conclusion, both temperature and the solvent in which the compounds are evaporated impact on the recovery of compounds. When looking at trends with regards to temperature, the highest recovery for almost all compounds was achieved at 20 ˚C. Temperature was found to be most influential in basic methanol, with temperature effects less apparent in methanol. Although it cannot be said with certainty, it is assumed that this is due to the pH in basic solvent being higher than the pKa of basic analytes. The analytes are consequently non-ionised and more non-polar which will increase their volatility. However, evaporation at 20 ˚C leads to a time-consuming evaporation step. In this study, to compromise between time taken for evaporation and recovery of compound, an evaporation temperature of 40 ˚C was selected. Solvent used for evaporation can have a significant impact on the recovery of compounds and should be considered when evaluating an SPE procedure. As previously mentioned, within published methodologies the evaporation temperature typically used is 35 – 40 ˚C in either methanol or basic methanol. This means it is likely that loss of compounds to some extent would have occurred in all these procedures.

3.2 Effect of silanisation of glassware

In trace analysis, loss of compounds due to adsorption onto glassware surfaces can be significant [15]. Untreated glassware contains silicate and silanol groups that act as ion-exchange and nucleophilic centres [16]. Consequently, compounds may be lost where they come into contact with glassware, such as during sample collection and evaporation of SPE extracts. Amines are especially prone to loss onto the slightly acidic surface of glass [17]. To prevent the loss of compounds, glassware used for trace analysis can be silanised or ‘deactivated’. Silanisation involves masking the polar Si-OH groups and decreasing its hydrophilicity by chemically binding a non-adsorptive silicone layer on to the surface of the glassware. Ahrer et al. [18] investigated the loss of 27 endocrine disrupting compounds and PPCPs in both water and SPE extracts and found ‘remarkable’ improvement in recoveries after silanisation. However, recovery values were not reported in the manuscript.

To assess the influence of non-silanised and silanised glassware during the evaporation step, methanol (3 mL) and basic methanol (3 mL, pH 11.8), were spiked at environmentally realistic concentrations (50 ng of each compound) and evaporated at 40 ˚C which therefore simulated the drying of SPE extracts in Oasis HLB and MCX elution solvents. Recoveries were calculated against spiked sample diluent at the same spiking level.

The results are listed in Table 2 and show several significant improvements in recovery between non-silanised and silanised vials in both solvents, with the majority of increases observed in basic methanol. Cocaine reported a 34 % increase in recovery between non-silanised and silanised vials in basic methanol, whereas in methanol recovery was high in both types of glass. Similar results were

reflected in that of norcocaine and cocaethylene. On the other hand, cocaine metabolites benzoylecgonine and norbenzoylecgonine showed high recoveries in both vials and solvents. Anhydroecgonine methyl ester presented an increase in recovery when vials were deactivated of 47 and 46 % in basic methanol and methanol, respectively. Surprisingly, ecgonidine reflected the opposite trend, with lower recoveries in the silanised vials as opposed to the non-silanised.

The suite of amphetamine-like compounds showed exceptional increases in recovery with the deactivation of vials in basic methanol. Increases were also observed in methanol, although not to such a large extent. For example in basic methanol, respective amphetamine and methamphetamine recoveries were 16 and 23 % in non-silanised vials, with these figures increasing to 88 and 89 % respectively in silanised vials. Recovery of LSD showed minor improvement with silanisation, whereas its main metabolite 2-oxo-3-hydroxy-LSD reported an improvement of 13 and 16 % when vials were silanised in basic methanol and methanol respectively.

The silanisation of vials had little effect on the recovery of heroin or 6-acetylmorphine in both solvents. Similar results were also evident for many other opioids including oxycodone, buprenorphine, and fentanyl. Interestingly, a number of compounds in this group showed little change in basic methanol, whereas a decrease in recovery was observed in the case of deactivated vials in methanol only. This was the case for the following compounds: codeine, norcodeine, morphine, normorphine and dihydrocodeine. Methadone and its metabolites EDDP and EMDP all showed significant improvements when vials were silanised in basic methanol. Most notably the recovery of EDDP increased from 16 % in non-silanised vials to 89 % in silanised vials.

In general there appeared to be little influence on the benzodiazepines through silanisation. The same was also found for antidepressants, with exception of venlafaxine which was found to increase with the silanisation of glassware in basic methanol. Ketamine and norketamine increased significantly with the silanisation of glass in both solvents. Ketamine recovery increased from 20 % in basic methanol to 84 % when vials were deactivated.

Given the potential for loss of analytes on glassware without silanisation, it is surprising that only three published manuscripts in relation to drugs of abuse in the environment mention the silanisation of their glassware. The remaining 16 papers do not report whether or not deactivation has been carried out. Of these 16 papers, four have reported sample preparation recoveries decoupled from matrix effects. A review of these four papers for the recovery of amphetamine (one of the most influenced compounds by silanisation) showed recoveries of 70 % [19], 102 % [20], 90 % [21] and 74 % [22]. It is difficult to interpret these results with regards to silanisation as the deactivation of glassware was not reported and there are several factors which may have affected recovery. Nevertheless, it is possible that the lower amphetamine recovery reported in two of these manuscripts was due to glassware not being deactivated.

The results clearly demonstrate that for many of the compounds studied in this investigation, the silanisation of glassware used to evaporate extracts provides improvements in recovery. The results suggest deactivation was more influential in basic methanol as opposed to methanol. The most significant improvements were observed for amphetamine type compounds and methadone and associated metabolites. This study can conclude that all glassware should be silanised that is used during the evaporation step. Furthermore, there is the possibility that all glassware that comes into contact with the sample could adsorb certain compounds; hence all glassware throughout the sample preparation should be silanised.

3.3 Stability studies

Improving understanding of the stability of drugs of abuse in the environment is crucial for several reasons [23]: currently no uniform way of sample collection and handling exists; few stability studies have been conducted in wastewater; the majority of pharmaceuticals and drugs of abuse are bioactive

and hence may be metabolised or degraded by bacteria in wastewater or by other transformation reactions. Thus, depending on stability, quantifying a compound in wastewater that has been excreted several hours previously may in fact lead to a significant over or under estimation of the actual amount of residue originally present.

The stability of drugs of abuse in wastewater to date has not been comprehensively addressed. Castiglioni et al. [24] investigated the stability of several illicit drugs and metabolites in raw wastewater influent stored at 4 ˚C with samples analysed immediately after spiking and three days. (pH not mentioned, although this would be assumed to be around pH 7, the normal pH of wastewater influent). This was the first publication to address the stability of illicit drugs in wastewater, although this work was limited due to only one storage condition and one time point being addressed. Gheorghe et al. [25] followed up this work by monitoring the stability of cocaine and benzoylecgonine in pond water, with pH (2 and 6) and temperature (-20, 4 and 20 ˚C) modified and time-points of 24, 72, and 120 hours. In the same publication, the stability of cocaine, benzoylecgonine and ecgonine methyl ester were assessed in wastewater influent at 20 ˚C and pH 6 (as the authors intention was to recreate the conditions present in the environment this wastewater was presumably unfiltered, although this information is not mentioned in the manuscript). This publication provided evidence for the preservation of cocaine at an acidic pH. However, the drawback of this work was the small number of compounds monitored and the use of pond water rather than wastewater in all but one study. More recently, González-Marino et al. [26] monitored the stability of several illicit drugs in filtered wastewater in the dark at 4 ˚C. The same experiment was also duplicated with the addition of a preserving agent NaN_3 (0.2 %). Target analytes were spiked at 100 µg L^{-1} with stability monitored at 1, 3, 5 and 7 days. In the same study, wastewater was spiked and extracted onto Oasis HLB cartridges which were then stored at -20 ˚C for 12 weeks. The addition of a concentration of 100 µg L^{-1} of each compound is a cause for concern in this work as such a high concentration is environmentally unrealistic.

The various storage conditions tested in this study recreate many of the conditions encountered during environmental analysis. The most commonly employed method for the collection of wastewater is through the use of a 24 hr (often temperature controlled) composite sampler (see Table 1). The various methods of sample collection were well-discussed by Ort et al. [27], although this article did not mention potential stability issues. Target analytes will also be present in wastewater for a certain period of time (often hours) as the sample travels through the sewage system to the WWTP composite sampler. Further still, the sample then has to be transported to the laboratory where it may then be kept at a refrigerated temperature for up to three days [1] before analysis. Consequently, to assess stability, untreated raw (unfiltered) wastewater influent was spiked with target analytes and stored at 2 and 19 ˚C and analysed over a period of three days.

Acidification of samples is widely known to prevent bacterial activity, and in turn preserves the sample [28]. Gheorge et al. [25] investigated the stability of cocaine and principal metabolites in surface water at different pH values (2 and 6) and temperatures (-20, 4 and 20 ˚C). It was found that storage of samples at an acidic pH was the primary factor in improving stability. Acidification of samples is also a requirement if basic molecules are to be extracted by SPE with mixed mode cation exchange sorbents; hence the pH of samples does need to be adjusted. For these reasons the stability of compounds was assessed in acidified (pH 2) wastewater (filtered) stored at 2 and 19 ˚C.

A further set of conditions assessed was the storage of analytes in filtered wastewater as opposed to unfiltered wastewater. The removal of suspended wastewater particulates may improve the stability of analytes as it prevents potential adsorption onto particulates. For this reason filtered wastewater was spiked with analytes and stored at 2 and 19 ˚C.

A control sample (not spiked) was analysed alongside each of the spiked samples. The stability patterns for compounds present in the control sample were very similar to those in the spiked sample, although often the change (either increase or decrease) was more severe in the control sample than the spiked sample. As the ratio of compound to bacteria in the control samples was lower, this may be

one possible reason to account for the difference. As both the control and spiked samples reported similar degradation patterns, it was decided that spiking wastewater at 1000 ng L^{-1} (in addition to the concentration already present in the control sample, see Table S4) would still provide an accurate estimate of stability. The stability of all compounds in raw (unfiltered) wastewater are listed in Table 3, acidified filtered wastewater in Table 4 and filtered wastewater at pH 7 in Table 5.

In the stability tests performed in the present study, benzoylecgonine reported a high stability in all storage conditions, although a slight increase in its concentration was shown when stored at an unadjusted pH. The increase in benzoylecgonine was likely due to the hydrolysis of cocaine to benzoylecgonine [25]. These results are in good agreement with previously reported findings [24-26]. With regards to cocaine, as was the case in the work by Gheorghe et al. [25], the main factor influencing the stability was found to be pH, with stability significantly improved at an acidic pH. Stored in filtered wastewater at pH 2 and 7 (both at 19 ˚C) the stability of cocaine after three days was found to decrease by 6.4 and 28.3 % respectively. In contrast to previous authors findings [24-26], the degradation of cocaine in this study was shown to be less severe at a natural pH. For example, after three days in raw (unfiltered) wastewater stored at 2 ˚C, a stability decrease of 8.2 % was found in contrast to Castiglioni et al. [24] of 36.1 %. More severe still, Gheorghe et al. [25] reported a reduction in cocaine of nearly 90 % after 24 hours in ambient temperature at pH 6. The contrast in findings could be due to the wastewater used in each of these studies. All of these studies have been conducted in different countries, thus the composition of wastewater may vary significantly depending on factors such as inhabitants, industrial input and time of year. Cocaine metabolites norcocaine, norbenzoylecgonine and cocaethylene all showed good stability in the various storage conditions in filtered water. After three days, in raw (unfiltered) wastewater at pH 7 and stored at 2 ˚C, norcocaine, norbenzoylecgonine and cocaethylene reported a stability decrease of 14.4, 6.0, and 9.4 % respectively, which is good agreement with the findings by Castiglioni et al. [24] decreasing by 15.4, 13.0 and 13.0 %. However, after 72h storage of unfiltered wastewater at 19 ˚C a significant degradation of these compounds was observed (31.5% and 19% for norcocaine and cocaethylene respectively). Crack cocaine compounds anhydroecgonine methyl ester and ecgonidine both showed good stability at pH 2 regardless of temperature with less than a 15 % change reported after 72 hours. However, when stored in filtered wastewater at pH 7 and at 19 ˚C these compounds reported significant increases in concentration, with an increase of 45.3 and 43.3 % reported for anhydroecgonine methyl ester and ecgonidine, which suggests transformation processes occurring.

With regards to amphetamine like compounds, in general, these analytes showed good stability in all storage conditions, especially when stored in acidic wastewater. After 72 hours in raw (unfiltered) wastewater at pH 7 and at 2 ˚C, methamphetamine reported a decrease of 6.0 % which agrees well with the value of 0.3 % determined by Castiglioni et al. [24]. Similarly, the stability of MDA and MDMA in the same sample in this study reported a change of 3.5 and 3.1 % which again compares well with Castiglioni et al. [24] of 4.4 and 0.8 %. In contrast, amphetamine reported a significant upsurge in concentration, which differs to that of Castiglioni et al. [24] and Gonzalez-Marino et al. [26] with both reporting good stability of amphetamine. As previously mentioned, the stability of methamphetamine was in good agreement with Castiglioni et al. [24], although in contrast to these two studies, Gonzalez-Marino [26] reported almost complete degradation of methamphetamine after three days. Methcathinone, MBDB and BDB showed good stability in wastewater at pH 2, but their stability was significantly deteriorated at pH 7, especially in the case of methcathinone, which after three days at pH 7 and at 2 ˚C reported a stability decrease of -60.3 %.

Heroin reported a dramatic decrease in stability in all samples stored at pH 7 in this study, as opposed to an acidic pH where the decrease was less than 15 % after three days. A similar pattern, although not to the same extent, was observed for 6-acetylmorphine. After three days in filtered wastewater at pH 7 and at 2 ˚C, heroin reported a decrease of 68.4 %, which is in good agreement with Gonzalez-Marino et al. [26] who showed a decrease of around 80 %. The stability of heroin and 6-acetylmorphine was adversely affected through the presence of suspended solids. After 24 hours in filtered waster at pH 7 and at 2 ˚C, heroin and 6-acetylmorphine reported a stability decrease of 38.7 and 11.0 % respectively, whereas in the presence of suspended solids at the same storage conditions their

respective stabilities decreased by -82.3 and -32.5 %. Furthermore, only 12h storage at neutral pH (temp, 2 ˚C) led to a 66% decrease in concentration of heroin. This outcome questions currently used 24h-sampling as a technique of choice in sewage epidemiology, where accurate measurement of heroin and its metabolites in wastewater is critical in order to estimate heroin's abuse.

Morphine and its glucuronide conjugate showed good stability at an acidic pH, whereas in all samples stored at pH 7, morphine reported an increase between 45.3 and 89.1 % after three days. This significant increase of morphine was paralleled by the decrease in the stability of morphine-3β-glucuronide. These findings are supported by the results of Castiglioni et al. [24], who attributed the decrease of the conjugate to its deconjugation by the β-glucuronidase enzymes of faecal bacteria. In raw (unfiltered) wastewater at pH 7 and at 2 ˚C, after 12 hours morphine reported an increase of 46.2 % and its conjugate reported a decrease of 80.5 %. Normorphine was determined to be stable in all conditions assessed. Codeine, norcodeine, oxycodone, dihydrocodeine, buprenorphine and norbuprenorphine were all found to be relatively stable in all storage conditions, with stability in raw (unfiltered) wastewater at 19 ˚C of less than 17.4 %. Methadone and its primary metabolite EDDP after three days in raw wastewater at 2 ˚C showed a decrease of 10.9 and 17.1 %, which is close to the respective values reported by Castiglioni et al. [24] of 5.2 and 1.6 %.

In contrast to all of the other compounds targeted in this study, benzodiazepines temazepam and oxazepam showed lower stability in acidic wastewater as opposed to pH 7, with stability at pH 2 and at 19 ˚C after three days decreasing by 62.7 and 29.4 %. Stability was improved in the same sample through a cooler storage temperature of 2 ˚C to provide a decrease in stability at the same time point of 23.4 and 8.6 %. In contrast, the remaining benzodiazepines, diazepam, nordiazepam, nitrazepam, 7-aminonitrazepam and chlordiazepoxide showed greater stability when the wastewater was acidified. The stability of nitrazepam shows a particularly sharp decline when stored in filtered wastewater at pH 7 and at 19 ˚C, with a stability decrease of 77.2 % after 24 hours. The stability of this compound was improved through acidification, although even when wastewater was acidified the stability of nitrazepam still decreased by 35.1 % after three days at pH 2 and at 2 ˚C. The above suggests that transformation processes occur in wastewater even in a short timescale of 12h and raises a question of the validity of composite sampling in the analysis of these compounds in wastewater. A decrease of concentrations of some benzodiazepines and an increase in concentrations of others might simply result from the fact that benzodiazepines share common metabolism patterns and additionally they are often excreted as glucuronic acid conjugates, which might be broken down with the formation of free compounds.

The stability of antidepressants dosulepin, fluoxetine, amitriptyline and venlafaxine in this study was found to decrease by 25.4, 15.1, 23.6 and 15.1 % respectively, when stored at pH 2 and at 2 ˚C for three days in filtered wastewater. Furthermore, a significant decrease of their concentrations was observed unfiltered wastewater stored at pH 7.4 and at 2 ˚C for 72h and denoted 72.3, 54.8, 61.2 and 43.6% for dosulepin, fluoxetine, amitriptyline and venlafaxine respectively. Nortriptyline and norfluoxetine showed poor stability in all storage conditions, for instance stability in acidified wastewater at 2 ˚C was 32.6 and 36.4 % respectively after three days. Ketamine and its metabolite were found to have excellent stability with very little change in stability after three days in all storage conditions tested.

Table 6 summarises each of the time points in the various storage conditions by showing the percentage of compounds (65 compounds were analysed in total) reporting a stability change greater than 15 %. The table shows clearly that after 12 hours nearly all compounds are stable at an acidic pH, regardless of temperature, in contrast to wastewater at pH 7 with between 23 - 35 % of compounds reporting a significant change in stability. After 24 hours, nearly half of all the compounds monitored reported a stability change at neutral pH, in comparison to acidified wastewater with only 8 % of analytes. After 72 hours, stability was especially poor in raw (unfiltered) wastewater at 19 ˚C with 69 % of compounds reporting a significant stability change.

Concerning the various parameters modified in the study, pH was the most important factor with a significant improvement in the stability of compounds overall when wastewater was acidified. This was also the finding of Gheorghe et al. [25] for cocaine and benzoylecgonine. Storage temperature of samples was also found to affect stability, although to a lesser extent than pH. After three days in raw (unfiltered) wastewater at temperatures of 2 and 19 ˚C the percentage of compounds reporting a significant stability change were 54 and 69 % respectively. A similar increase in stability was also observed when storing samples at 2 ˚C for filtered wastewater at pH 7. In contrast, acidified wastewater actually presented slightly better stability at a temperature of 19 ˚C, although this difference was minimal at 8 % between the two temperatures. The influence of suspended solids in the samples was found to decrease concentrations of analytes in the aqueous matrix, with the percentage of compounds reporting a concentration change in raw (unfiltered) wastewater at 19 ˚C of 69 % in comparison to filtered wastewater of 54 %. These findings are of critical importance when assessing validity of the usage of composite sampling and indicate the need for re-evaluation of current sampling approaches.

3.3.1 Long term storage stability

After the collection of environmental samples it is often necessary for the long term storage of samples (a few weeks), perhaps due to instrument issues or analyst time restrictions. The stability of several illicit drugs and metabolites in wastewater stored at -20 ˚C was investigated by Chiaia et al. [29]. This group reported the stability of compounds for three weeks. However, storage in this way can be problematic with a large number of samples due to space restrictions and the time required waiting for samples to thaw. González-Marino et al. [26] investigated the storage of compounds stored at -20 ˚C, after their extraction onto Oasis HLB cartridges, and found no stability issues.

In this study, spiked wastewater influent was extracted on to Oasis MCX cartridges, washed with 2 % $HCOOH/H_2O$, blow dried, and stored at -20 ˚C, with the exception of two cartridges which were eluted immediately and served as time point 0. Results are presented in Figure S1 as a normalised percentage against time point 0. Within the six weeks evaluated no degradation of compounds was experienced, therefore this method of storage is ideal for the multi-class compounds studied and may be a more suitable method than freezing large volumes of aqueous samples.

4 CONCLUSION

In conclusion, for the first time a comprehensive stability study of >60 drugs of abuse has been presented over a period of three days in an environmental matrix. The data shows that from the perspective of stability, composite samplers are unsuitable with regards to some compounds. For instance heroin and 6-acetylmorphine reported a decrease in stability of 66 and 26 % respectively after only 12 hours in raw (unfiltered) wastewater at 2 °C. Acidification of samples was found to improve stability for the majority of compounds. One possible way to improve stability would be through the addition of a small volume of acid over regular intervals during a 24 hr composite sample collection. It has to be however remembered here that acidification might influence partitioning of certain drugs between aqueous solution and (suspended) solids. If this is the case both aqueous solution and solids have to be analysed for the presence of studied compounds.

This manuscript has also presented results for several previously underreported parameters. A summary of the range of recoveries from each studied parameter are presented in Table 7. This table, through colour coordination, clearly demonstrates the most critical factors that affect recovery are stability, silanisation of glassware, and the solvent and temperature used during the evaporation of SPE extracts. Silanisation of glassware used during the evaporation of SPE extracts was found to significantly improve recoveries of some compounds, especially amphetamine type compounds. The elution solvent was found to affect recoveries, for instance in the case of heroin which achieved a

lower recovery when dried in basic methanol as opposed to methanol. The evaporation temperature caused the loss of some compounds, and for the majority of compounds should be no higher than 40 °C. To summarise, the findings of the above presented research clearly indicate the need for more rigorous reporting of method validation data (including sample collection, preservation and preparation) as these often underreported parameters might have major impact on the overall method performance.

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SUPPLEMENTARY MATERIAL

Supplementary material includes:

Figure S1. Stability of analytes on Oasis MCX cartridges, extracted from the spiked WWTP influent, and stored at -20 ˚C over a 6 week period (n = 2 at each time-point)

Figure S2. SPE sorbent evaluation, total recovery of Oasis HLB and MCX for all compounds from UHQ water (n = 3)

Table S1. Selected pharmaceuticals and their properties

Table S2. Absolute recovery of analytes in basic methanol after the evaporation of solvent

Table S3. Absolute recovery of analytes in methanol after the evaporation of solvent

Table S4. Stability study - concentration of compounds in control wastewater sample at time-point zero

Table S5. Filter membrane investigation (recoveries < 90 % highlighted)

Table S6. Recovery of analytes after filtration through glass fibre filters

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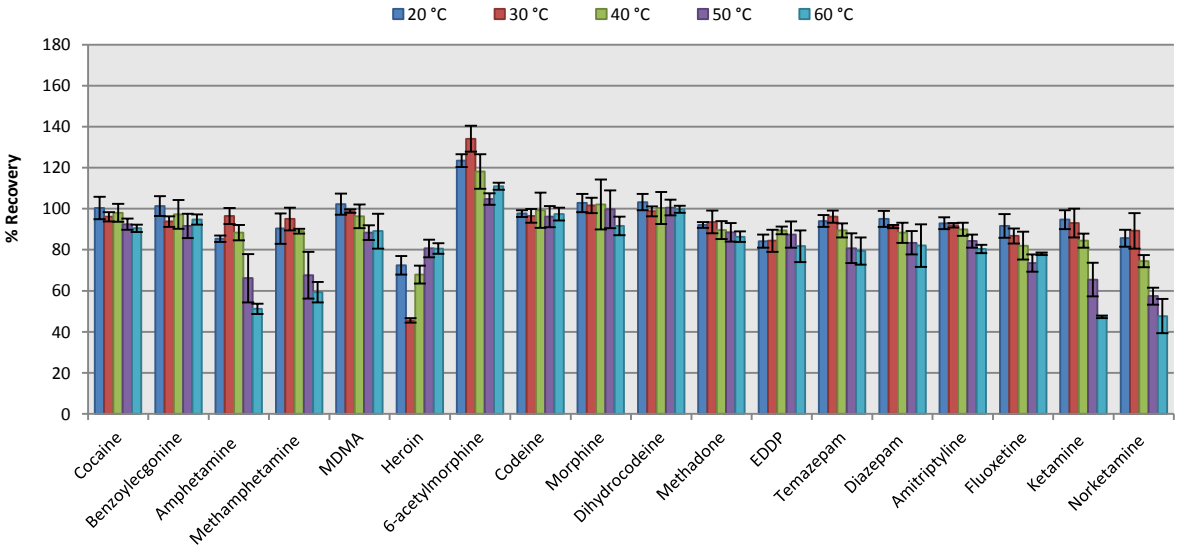
FIGURES

Figure 1 – Recovery (n = 3) of selected compounds after evaporation temperatures 20, 30, 40, 50 and 60 °C from a) 7% NH_4OH in methanol and b) methanol



B)

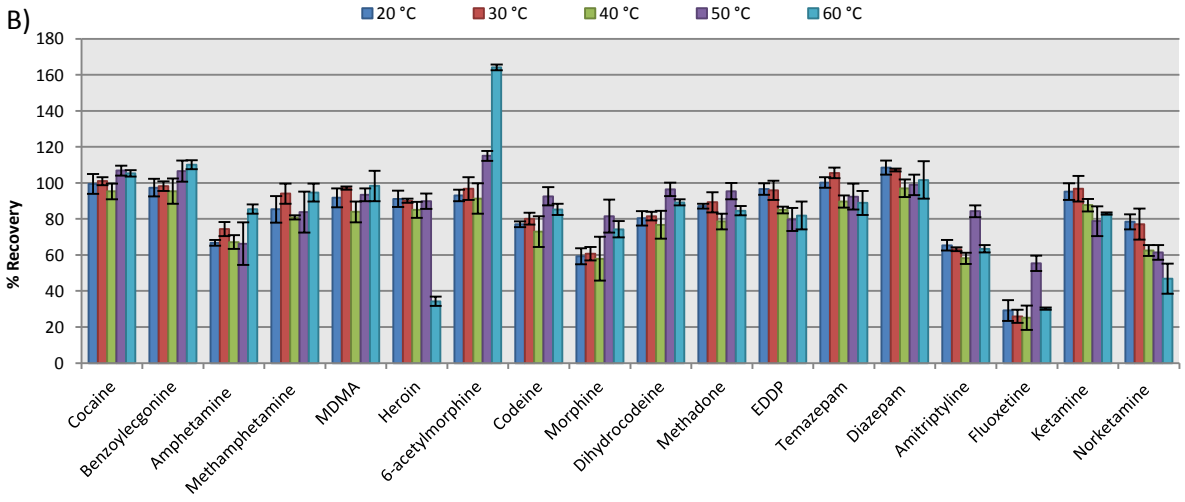


Table 1 – Sample preparation and analysis reported for the determination of drugs of abuse and related compounds in the environment

Analytes	Sample collection	Storage and preparation	Extraction	Sample volume; enrichment factor	SPE extract evaporation and reconstitution	Absolute recovery (%)	Pre LC-MS filter	Separation-ionisation-detection (acquisition mode)	Method: MDL, MQL (ng L^{-1})	Observations	Ref.
2 x ALC, 1 x antidepressant	POCIS – 28 to 30 days deployment	Not reported	Polar organic chemical integrative sampler (POCIS)	Not reported	Extract concentrated (procedure not reported)	Not reported	None reported	RP-HPLC-ESI(+)-ITMS (Full scan and SIM)	Water: (IDL[f]) MAMP, 0.19; MDMA, 0.25; FLU, 20	• First paper reporting analytical method for the detection of illicit drugs • Full scan results confirmed with CID • Silanisation of glassware not reported	Jones-Lepp et al. [30]
5 x cocainics, 5 x ALC, 5 x opiates, 1 x cannabinoid	24 h composite samples - collected every 20 min	Samples stored at 4 ˚C and extracted within 3 days. Samples filtered through 1.6 µm glass microfiber filters	SPE - Oasis MCX 60 mg Condition: 6 mL MeOH, 3 mL H_2O, 3 mL H_2O (pH 2) Wash: None Elution: 3 mL MeOH, 3 mL MeOH:2 % NH_3	WWinf: 50 mL (pH 2); 250 x WWeff: 50 mL (pH 2); 250 x	Extract evaporated (temperature not reported). Reconstituted in 200 µL H_2O.	WWinf[c]: COC, 96±5; BE, 107±9; COE, 109±4; nor-COC, 112±7; nor-BE, 85±5; AMP, 110±5; MAMP, 112±7; MDA, 102±3; MDMA, 104±2; MDEA, 107±4; MOR, 88±7; M3G, 90±11; 6ACM, 106±5; MET, 112±7; EDDP, 88±3; THC-COOH, 51±1 WWeff[c]: COC, 94±4; BE, 107±2; COE, 98±2; nor-COC, 119±2; nor-BE, 104±3; AMP, 103±4; MAMP, 97±3; MDA, 98±3; MDMA, 97±3; MDEA, 89±3; MOR, 75±3; M3G, 67±0; 6ACM, 93±8; MET, 90±2; EDDP, 85±4; THC-COOH, 61±4	Samples centrifuged	RP-HPLC-ESI(+/-)-QqQ (2 SRM)	WWinf: (MQL) COC, 1.4; BE, 1.98; COE, 0.95; nor-COC, 1.92; nor-BE, 0.94; AMP, 5.4; MAMP, 3.7; MDA, 8.7; MDMA, 6.3; MDEA, 4.19; MOR, 3.95; M3G, 0.63; 6ACM, 5.3; MET, 1.14; EDDP, 1.64; THC-COOH, 1.75 WWeff: (MQL) COC, 0.99; BE, 0.92; COE, 0.66; nor-COC, 0.67; nor-BE, 0.56; AMP, 2.8; MAMP, 1.11; MDA, 2.6; MDMA, 1.74; MDEA, 1.64; MOR, 3.2; M3G, 0.48; 6ACM, 3.08; MET, 0.97; EDDP, 0.6; THC-COOH, 0.94	• The first 'comprehensive' analytical method for drugs of abuse in the environment • Stability studies • Internal standard quantification (15 deuterated standards) • Silanisation of glassware not reported	Castiglioni et al. [24]
1 x cocainics, 6 x opiates, 4 x benzodiazepines (+9	Grab samples	Samples stored at 4 ˚C and extracted within 1 day. Sample	SPE - Oasis HLB 200 mg Condition: 2 mL n-heptane, 2 mL acetone, 3	WWinf: 100 mL; 200 x	Extracts evaporated to approx. 50 µL and made up to	WWinf[a]: BE, 40±5; COD, 48±2; DICOD, 36±1; MET, 44±2; MOR, 29±1; OXYC, 50±5; TRAM, 20±1; DIAZ, 18±1; nor-DIAZ, 18±3; OXAZ, 42±5; TEM, 19±7	None reported	RP-HPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) BE, 10; COD, 20; DICOD, 20; MET, 50; MOR, 10; OXYC, 20; TRAM, 20; DIAZ, 10; nor-DIAZ, n.r; OXAZ, 20; TEM, 10	• Matrix effects evaluated: WWinf[e]: 28 - 90 %; WWeff[e]: 10 - 45 % ; RW[e]: 3 - 20 % • Internal standard	Hummel et al. [31]

additional compounds)		s filtered through < 1 µm glass fibre filters	x 3 mL MeOH, 4 x 2 mL water (pH 7) Wash: None Elution: 4 x 2 mL acetone	WWeff: 200 mL; 400 x RW: 1000 mL; 2000 x GW: 1000 mL; 2000 x DW: 1000 mL; 2000 x	approx. 500 µL H_2O:10 % MeOH	WWeff[a]: BE, 42±2; COD, 64±6; DICOD, 70±5; MET, 77±2; MOR, 43±8; OXYC, 69±10; TRAM, 49±15; DIAZ, 61±2; nor-DIAZ, 69±2; OXAZ, 61±7; TEM, 57±4 RW[a]: BE, 70±5; COD, 76±5; DICOD, 92±5; MET, 83±7; MOR, 86±8; OXYC, 86±6; TRAM, 74±9; DIAZ, 84±5; nor-DIAZ, 93±3; OXAZ, 93±9; TEM, 78±4 GW[a]: 71 - 123 %, DW[a]: 25 - 102 %,			WWeff: BE, 5; COD, 10; DICOD, 10; MET, 25; MOR, 5; OXYC, 10; TRAM, 10; DIAZ, 5; nor-DIAZ, n.r,; OXAZ, 10; TEM, 5 RW: BE, 1; COD, 2; DICOD, 2; MET, 5; MOR, 1; OXYC, 2; TRAM, 2; DIAZ, 1; nor-DIAZ, n.r.; OXAZ, 2; TEM, 1	quantification (8 deuterated standards) • Silanisation of glassware not reported	
2 x cocainics, 5 x ALC, 2 x dissociative anesthetics, 1 x lysergic, 1 x opiate, 4 x urine indicators,	24 h composite samples at WWTP; grab samples from RW	Samples stored at 4 °C and extracted within 1 day. Samples filtered through 1.6 µm glass microfiber filters	SPE - Oasis HLB 200 mg Condition: 10 mL MeOH, 10 mL H_2O Wash: 8 mL H_2O:5 % MeOH Elution: 6 mL MeOH	WWinf: 100 mL; 200 x WWeff: 100 mL; 200 x RW: 100 mL; 200 x	Extract evaporated at 35 °C. Reconstituted in 500 µL H_2O:5 % MeOH	WWinf[b]: COC, 86±6; BE, 92±6; AMP, 70±7; MAMP, 80±4; MDA, 74±5; MDEA, 101±5; MDMA, 88±6; LSD, 75±5; KET, 85±5; PCP, 85±4; FENT, 80±4; CAFF, 83±6; PARAX, 71±6; NIC, 80±5; COT, 78±6 RW[b]: COC, 90±4; BE, 95±4; AMP, 75±4; MAMP, 83±2; MDA, 75±4; MDEA, 99±3; MDMA, 90±5; LSD, 7±4; KET, 85±5; PCP, 88±2; FENT, 82±3; CAFF, 85±5; PARAX, 80±5; NIC, 82±3; COT, 81±4	0.2 µm filter[g]	RP-UPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) COC, 0.2; BE, 0.2; AMP, 1; MAMP, 0.9; MDA, 1.0; MDEA, 2.1; MDMA, 1.5; LSD, 2.0; KET, 5.0; PCP, 2.0; FENT, 4.0; CAFF, 5.0; PARAX, 850; NIC, 800; COT, 500 RW: (MQL) COC, 0.12; BE, 0.12; AMP, 0.45; MAMP, 0.41; MDA, 0.43; MDEA, 0.43; MDMA, 0.52; LSD, 0.71; KET, 0.47; PCP, 0.54; FENT, 0.98; CAFF, 0.96; PARAX, 72.2; NIC, 103.0; COT, 83.9	• Stability of extracts investigated; Compounds stable in methanol extract at -20 °C and 4 °C (except paraxanthine) for 7 days. • Internal standard quantification (13 deuterated standards and ^{13}C-Caffeine) • Silanisation of glassware not reported	Huerta-Fontela et al. [19]
9 x opiates, 2 x cannabinoids	24 h composite samples	Samples stored at 4 °C and extracted within 3 days. Samples filtered through 1.6 µm glass microfi	SPE - Oasis HLB 200 mg Condition: 5 mL MeOH, 5 mL H_2O Wash: 3 mL H_2O Elution: 8 mL MeOH	WWinf: 200 mL; 400 x WWeff: 200 mL; 400 x	Extracts evaporated at 40 °C to 500 µL. One aliquot (250 µL) removed (for cannabinoids). The	WWinf[c]: HER, 65; 6ACM, 90; MOR, 83; nor-MOR, 95; COD, 94; nor-COD, 89; MET, 75; EDDP, 68; FENT, 72; THC, 42; THC-COOH, 96 WWeff[c]: HER, 75; 6ACM, 95; MOR, 99; nor-MOR, 86; COD, 85; nor-COD, 94; MET, 97; EDDP, 69; FENT, 95; THC, 44;	0.2 µm GHP filter	RP-UPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) HER, 20.0; 6ACM, 3.1; MOR, 7.1; nor-MOR, 25.0; COD, 2.5; nor-COD, 5.0; MET, 0.3; EDDP, 0.7; FENT, 1.7; THC, 8.3; THC-COOH, 12.5	• Cannabinoids injected in a separate methanol fraction to ensure resolution and sensitivity • Internal standard quantification (9 deuterated standards) • Silanisation of	Boleda et al. [32]

		ber filters		RW: 200 mL; 400 x	second aliquot (250 µL) evaporated to dryness and reconstituted in H_2O (volume not specified).	THC-COOH, 86			RW: (MQL) HER, 1.5; 6ACM, 0.9; MOR, 2.9; nor-MOR, 12.5; COD, 0.5; nor-COD, 1.6; MET, 0.1; EDDP, 0.4; FENT, 0.4; THC, 7.0; THC-COOH, 10.0	glassware not reported	
3 x cocainics, 1 x ALC, 3 x opiates, 1 x lysergic, 1 x antidepressant 2 x benzodiazepines	24 h composite samples at WWTP; Grab samples from RW	Samples extracted within 1 day. Samples filtered through 1.2 µm glass microfiber filters	SPE - Strata-XC 200 mg Condition: 2 x 6 mL MeOH, 2 x 6 mL H_2O Wash: 50 mL H_2O:10 % MeOH, 100mM HCOOH Elution: 10 mL 5 % (v/v) NH_4OH in 1:1 acetone:ethyl acetate	WWinf: 500 mL (pH 6.0); 2500 x WWeff: 500 mL (pH 6.0); 2500 x RW: 500 mL (pH 6.0); 2500 x	Extract reduced in volume with heating. (temperature not reported) Reconstituted in 200 µL H_2O:30 % MeOH (v/v), 5 mM CH_3COONH_4 (pH 4.5)	RW[a]: COC, 56±2; BE, 53±3; COE, 65±3; MDMA, 52±1; MOR, 4±0; MET, 55±0; EDDP, 59±2; LSD, 51±3; KET, 51±3; FLU, 33±2; TEM, 59±3; DIA, 55±3	None reported	RP-HPLC-ESI(+)-ITMS (1 SRM)	RW: (MQL) COC, 2; BE, 2; COE, 5; MDMA, 22; MOR, 856; MET, 14; EDDP, 7; LSD, 10; KET, 4; FLU, 312; TEM, 23; DIA, 127 (MDL) COC, 1; BE, 1; COE, 1; MDMA, 7; MOR, 257; MET, 4; EDDP, 2; LSD, 3; KET, 1; FLU, 93; TEM, 7; DIA, 38	• Cocaine stability investigated • Internal standard quantification (1 internal standard; papaverine) • Silanised glassware	Bones et al. [10]
3 x cocainics, 4 x ALC, 3 x lysergics, 3 x opiates, 3 x cannabinoids	24 h composite samples	Samples filtered through 1 µm glass microfiber filters and 0.45 µm nylon filters. Samples then stored at - 20 °C	SPE - PLRPs and Oasis HLB (for cannabinoids) Condition: 1 mL AcN Wash: 1 mL H_2O Elution: LC mobile phase	WWinf: 5 mL WWeff: 5 mL RW: 5 mL	N/A - online SPE	WWinf[a]: COC, 59; BE, 8; COE, 52; AMP, 15; MAMP, 20; MDMA, 27; EPH, 15; LSD, 17; nor-LSD, 22; O-H-LSD, 11; MOR, 14; HER, 22; 6ACM, 21; THC, 9; nor-THC, 13; OH-THC, 37	None reported	RP-HPLC-ESI(+/-)-QLIT (2 SRM)	WWinf: (MQL) COC, 2.40; BE, 5.24; COE, 0.69; AMP, 0.92; MAMP, 0.75; MDMA, 2.93; EPH, 2.21; LSD, 0.89; nor-LSD, 1.81; O-H-LSD, 2.60; MOR, 5.97; HER, 2.07; 6ACM, 5.17; THC, 3.37; nor-THC, 1.13; OH-THC, 1.45 (MDL) COC, 0.18; BE, 0.67; COE, 0.07; AMP, 0.34; MAMP, 0.28; MDMA, 1.10; EPH, 0.78; LSD, 0.27; nor-LSD, 0.68; O-H-LSD, 0.97; MOR, 1.51; HER, 0.78; 6ACM, 1.94;	• On-line SPE • M6G and M3G not effectively extracted by any of the tested cartridges • Matrix effects evaluated: WWinf[c]: 45 - 95 % • Relative recovery: WWinf: 71 - 745 % • Internal standard quantification	Postigo et al. [33]

									THC, 1.26; nor-THC, 0.43; OH-THC, 0.54	(12 deuterated standards)	
3 x cocainics	24 h composite samples at WWTP; Grab samples from RW	Samples adjusted to pH 2 and stored at -20 ˚C. Samples filtered though qualitative filter paper followed by glass microfiber filter	SPE - Oasis HLB 500 mg Condition: 3 mL MeOH, 3 mL H_2O Wash: 3 mL H_2O:5 % MeOH Elution: 2 x 4 mL MeOH	WWinf: 100 mL (pH 6); 667 x RW: 500 mL (pH 6); 3333 x	Extract evaporated (temperature not reported). Reconstituted in 150 µL MeOH: LC mobile phase A (1:1, v/v) for RPLC and in 150 µL AcN/MeOH (3:1, v/v) for HILIC	TW[a]: COC, 96±6; BE, 92±2; EME, 73±5	Samples centrifuged	RP-HPLC-ESI(+)-ITMS (1 SRM)	WWinf: (MQL) RP-HPLC, COC, 4; BE, 2 HILIC-HPLC, COC, 0.5; BE, 1; EME, 20	• Stability studies for COC and BE • First methodology employing HILIC • Matrix effects evaluated: Win, < 10 %; RW, < 10 % • Internal standard quantification (3 deuterated standards) • Silanisation of glassware not reported	Gheorge et al. [25]
4 x cocainics, 7 x ALC, 3 x opiates, 3 x dissociative anesthetics, 2 x Lysergics, 1 x benzodiazepine, 3 x urine indicators	24 h composite samples - flow normalised 4 °C	Samples stored at - 20 °C and analysed within 3 weeks.	N/A - large volume direct injection	WWinf: 7 mL	N/A - large volume direct injection	N/A - large volume direct injection	Samples centrifuged	RP-HPLC-ESI(+)-QqQ (1 or 2 SRM)	Water: (MQL) AMP, 10.0; MAMP, 10.0; MDMA, 2.5; MDA, 2.5; MDEA, 5.0; MBDB, 5.0; EPH, 10.0; COC, 2.5; BE, 10.0; nor-COC, 2.5; nor-BE, 5.0; HYDC, 2.5; OXYC, 2.5; MET, 2.5; KET, 5.0; nor-KET, 5.0; 2-OXO-LSD, 5.0; LSD, 2.5; PCP, 5.0; FLUN, 2.5; COT, 250; CAFF, 250; CREA, 50000 (MDL) AMP, 1.5; MAMP, 1.5; MDMA, 1.0; MDA, 2.0; MDEA, 3.5; MBDB, 4.0; EPH, 2.5; COC, 2.0; BE, 1.0; nor-COC, 2.0; nor-BE, 2.5; HYDC, 2.0; OXYC, 2.0; MET, 2.0; KET, 4.0; nor-KET, 3.5; 2-	• Creatinine used to estimate local population • Large volume direct injection • Stability study • No loss of compound after centrifugation (95 % CI) • Internal standard quantification (16 deuterated standards)	Chiaia et al. [29]

									OXO-LSD, 2.5; LSD, 0.5; PCP, 2.5; FLUN, 1.5; COT, 4.5; CAFF, 6.0; CREA, 250		
2 x cocainics, 1 x ALC, 2 x opiates, 1 x antidepressant (plus additional PPCPs)	Grab samples	Samples adjusted to pH 2 and stored at 4 °C. Samples filtered through 2.7 µm and 0.7 µm GF/F glass microfiber filters	SPE - Oasis MCX 60 mg Condition: 2 mL MeOH, 2 mL H_2O:2 % HCOOH Wash: 2 mL H_2O:2 % HCOOH Elution: 2 mL MeOH, 2 mL MeOH:5 % NH_4OH	WWinf: 250 mL (pH 2); 500 x WWeff: 250 mL (pH 2); 500 x RW: 1000 mL (pH 2); 2000 x	Extracts evaporated at 40 °C. Reconstituted in 500 µL LC mobile phase A	WWinf[a]: COC, 43-47; BE, 61-69; AMP, 73-105, COD, 51-51; TRAM, 101-145; AMIT, 2-4 WWeff[a]: COC, 49 - 50 %; BE, 70-98; AMP, 72-109, COD, 64-86, TRAM, 98-144, AMIT, 1-2 RW: COC, 70; BE, 131; AMP, 91; COD, 75; TRAM, 76, AMIT, 37	0.2 µm PTFE filter	RP-UPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) COC, 1; BE, 1; AMP, 3; COD, 2; TRAM, 10; AMIT, 32 WWeff: (MQL) COC, 1; BE,1; AMP, 3; COD, 2; TRAM, 10; AMIT, 2 RW: (MQL) COC, 0.2; BE, 0.1; AMP, 1; COD, 0.3; TRAM, 3; AMIT, 0.3	• Multi-residie analysis of a wide range of PPCPs • Internal standard quantification (1 internal standard; phenacetin-ethoxy-1-^{13}C) • Silanised glassware	Kasprzyk-Hordern et al. [34]
5 x cocainics, 5 x ALC, 1 x cannabinoid	24 h composite samples	Samples stored at - 20 °C. Samples centrifuged	SPE - Oasis MCX 150mg Condition: 6 mL MeOH, 3 mL H_2O, 3 mL H_2O (pH 2) Wash: 5 mL H_2O:2 % NH_4 Elution: 8 mL MeOH:2 % NH_4	WWinf: 50 mL (5 x diluted) (pH 2); 50 x WWeff: 50 mL (pH 2): 50 x RW: 50 mL (pH 2); 50 x	Extract evaporated at 35 °C. Reconstituted in 1000 µL H_2O:10 % MeOH	RW[a, e]: COC, 92; BE, 90; COE, 88; nor-BE, 90; nor-COC, 82; AMP, 65; MAMP, 65; MDMA, 95; MDA, 90; MDEA, 88; THC-COOH, 68	None reported	RP-UPLC-ESI(+)-QqQ (3 SRM)	WWinf: (MDL) COC, 3; BE, n.e.; COE, 2; nor-BE, 1; nor-COC, 5; AMP, 54; MAMP, 7; MDMA, 18; MDA, 91; MDEA, 40; THC-COOH, 2500 WWeff: (MDL) COC, 2; BE, 0.3; COE, 1; nor-BE, 0.2; nor-COC, 2; AMP, 40; MAMP, 1; MDMA, 9; MDA, 88; MDEA, 9; THC-COOH, 500 RW: (MDL) COC, 0.8; BE, 0.05; COE, 0.3; nor-BE, 2; nor-COC, 2; AMP, 2; MAMP, 0.6; MDMA, 4; MDA, 17; MDEA, 0.5; THC-COOH, 30	• Matrix effects evaluated: RW[e], -30 - 50 % • Relative recovery: WWinf: 57 - 120 %; WWeff: 63 - 118 %; RW: 61 - 120 % • Internal standard quantification (9 deuterated standards) • Silanisation of glassware not reported	Bijlsma et al. [35]
non-target analysis (full scan screening	Not reported	Not reported	SPE - Oasis MCX 500 mg Condition: AcN, MeOH, H_2O (pH 2) (volumes not reported) Wash: H_2O (volumes not	WWeff: 1000 mL (pH 2); 2000 x SW: 1000 mL (pH 2); 2000 x DW: 1000 mL (pH 2); 2000 x	Extract evaporated at 45 °C to 250 µL. 250 µL of H_2O added to make a final volume of 500	Not reported	None reported	RP-HPLC-ESI(+/-)-QqQ (Full scan and MS^n)	Illicits not quantified	• Non target analysis • Illicits not quantified • Silanisation of glassware not reported	Hogenboom et al. [9]

			reported) Elution: 2.5 mL AcN, 2 x 2.5 mL H_2O:5 % NH_4OH		µL						
3 x cocainics, 3 x ALC, 3 x opiates	24 h composite samples - volume proportional	Samples adjusted to pH 2 at collection site and stored at -20 °C. Samples filtered through glass microfiber filters	SPE - Oasis MCX 60 mg Condition: 6 mL MeOH, 4 mL H_2O, 4 mL H_2O (pH 2) Wash: 3 mL H_2O Elution: 4 mL MeOH, 4 mL MeOH:5% NH_3	WWinf: 50 mL; 250 x SW: 50 mL; 250 x	Extract evaporated (temperature not reported). Reconstituted in 100 µL AcN and 100 µL AcN:H_2O (90:10, v/v), 5 mM CH_3CONH_4	SW[b]: COC, 102±6; BE, 87±3; EME, 35±3; AMP, 102±6; MAMP, 99±4; MDMA, 100±4; MET, 103±3; EDDP, 61±8; 6ACM, 92±4	Sample centrifuged	HILIC-HPLC-ESI(+)-QqQ (2 SRM)	SW: (MQL) COC, 1; BE, 1; EME, 2; AMP, 2; MAMP, 1; MDMA, 1; MET, 1; EDDP, 1; 6ACM, 2	• HILIC • Comparison of MCX and HLB • Matrix effects evaluated: SW, 12 - 51 % • Internal standard quantification (9 deuterated standards) • Silanisation of glassware not reported	Van Nuijs et al. [20]
5 x ALC	Grab samples	Samples stored at 4 °C until analysis. Samples filtered through glass fiber prefilters and 0.45 µm nitrocellulose filters	SPE - SupelMIP - Amphetamine 25 mg Condition: 1 mL MeOH, 1 mL H_2O (pH 8) Wash: 2 x 1 mL H_2O (pH 8), 1 mL H_2O:40 % AcN Elution: 2 x 1 mL MeOH:1 % HCOOH	WWinf: 50 mL; 500 x WWeff: 50 mL; 500 x	Extract evaporated (temperature not reported). Reconstituted in 100 µL 2% NH_3 in MeOH:H_2O (1:1)	Wwinf[b, e]: AMP, 90; MAMP, 75; MDA, 102; MDMA, 98; MDEA, 83 WWeff[b, e]: AMP, 75; MAMP, 85; MDA, 75; MDMA, 87; MDEA, 75	None reported	RP-HPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MDL) AMP, 2.7; MAMP, 0.8; MDA, 2.2; MDMA, 2.3; MDEA, 0.5	• Comprehensive evaluation of MIPs, MCX and HLB • Matrix effects evaluated: WWinf: 30 - 55 %; WWeff: 10 - 45 % • Relative recoveries: WWinf, 92 - 111 %; WWeff, 92 - 114 % • Internal standard quantification (5 deuterated standards) • Silanisation of glassware not reported	González-Mariño et al. [21]
3 x cocainics, 5 x ALC, 2 x cannabinoids, 4 x	Grab samples	Samples extracted within 1 day. Samples	SPE - Oasis HLB 200 mg Condition: 5mL ethyl acetate, 5 mL	WWinf: 100 mL (pH8.5); 500 x	Extract (1) evaporated at 25 ˚C. Extract (1) and	Not reported	None reported	GC-EI-ITMS	WWinf: (MQL) COC, 39.6; BE, 26.4; COE,19.8; AMP, 23.1; MAMP, 23.1; MDMA; 26.4, MDA, 23.1; MDEA, 39.6; HER, 49.5; MOR, 36.3;	• Stability studies • Requires 90 minute derivatisation step • Relative	González-Mariño et al. [26]

opiates		filtered through 0.45 µm glass microfibre filters and 0.45 µm nitrocellulose filter	acetone, 5 mL H_2O Wash: None Elution: 2 x extracts (1) 2 mL ethyl acetate (2) 8 mL acetone	WWeff: 200mL (pH 8.5); 1000 x RW: 500 mL (pH 8.5); 2500 x	(2) mixed. Sample concentrated to 100 µL and 100 µL MSFA (derivatisation agent) added				COD, 19.8; MET, 19.8; THC, 9.9; THC-COOH, 3.3 Wweff: (MQL) COC, 9.9; BE, 13.2; COE, 19.8; AMP, 9.9; MAMP, 23.1; MDMA, 19.8;MDA, 16.5; MDEA, 36.3; HER, 49.5; MOR, 36.3; COD, 13.2; MET, 19.8; THC, 9.9; THC-COOH, 3.3 RW: (MQL) COC, 3.3; BE, 13.2; COE, 9.9; AMP, 2.6; MAMP, 6.6; MDMA, 6.6; MDA, 6.6; MDEA, 6.6; HER, 42.9; MOR, 9.9; COD, 3.3; MET, 6.6; THC, 3.0; THC-COOH, 3.3	recovery: WWinf: 63.4 - 137.2 %; WWeff, 85.4 - 134.2 %; RW, 73.9 - 124.7 % • Internal standard quantification (12 deuterated standards) • Silanisation of glassware not reported	
12 x cocainics, 5 x ALC, 4 x opiates, 2 x urine markers,	24 h composite samples	Sample filtered through 1.2 µm glass microfibre filters. Sample split into two fractions. Fraction 1 - (1.2 L) Adjusted to pH 2. Fraction 2 - (100 mL) Adjusted to 0.07 % formic acid (v/v). Samples stored at -20 ˚C and analysed within 2 days	SPE - Strata XC 500 mg Condition: 10 mL MeOH, 10 mL H_2O (pH 2) Wash: 10 mL H_2O (pH 2), 10 mL H_2O:2 % HCOOH, 10 mL H_2O:5 % MeOH Elution: 10 mL MeOH:2 % NH_4OH	WWinf: 200 mL (pH 2); 133 x	Extract evaporated at 35 ˚C to 500 µL. Diluted with 1000 µL of 0.1 % HCOOH :H_2O	Water[e]: COC, 100; BE, 85; nor-BE, 70; COE, 103; nor-COC, 99; EME, 67; ECG, 19; EEE, 35; AEME, 68; AEC, 115; AMP, 63; MAMP, 80; MDMA, 60; MDEA, 55; MBDB, 62; MOR, 65; 6ACM, 100; OXY, 65; HYD,63; CREA, 50; COT, 65; pHYBE, 72; mOHBE, 80	None reported	RP-HPLC-ESI(+)-QqQ (1 SRM)	WWinf: Direct injection: (MDL) COC, 4;BE, 33; nor-BE, 35; COE, 4; nor-COC, 3; EME, 32; ECG, 800; EEE, 179; AEME, 35; AEC, 2500; AMP, 37; MAMP, 15; MDMA, 15; MDEA, 4; MBDB, 4; MOR, 670; 6ACM, 22; OXY, 10; HYD, 16; CREA, 8130; COT, 47; pHYBE, 18; mOHBE, 16 SPE-LC-MS/MS: (MDL) COC, 0.03;BE, 0.29; nor-BE, 0.37; COE, 0.03; nor-COC, 0.02; EME, 0.36; ECG, 33; EEE, 3.9; AEME, 0.38; AEC, 19; AMP, 0.43; MAMP, 0.14; MDMA, 0.19; MDEA, 0.05; MBDB, 0.05; MOR, 7.7; 6ACM, 0.16; OXY, 0.11; HYD, 0.19; CREA, 120; COT, 0.53; pHYBE, 0.18; mOHBE, 0.15	• Direct injection and SPE compared • Secondary LC method employed for confirmation • Matrix effects evaluated: WWinf[e]: Direct inj., -15 - 65 %; SPE-LC-MS/MS: -5 - 72 % • Internal standard quantification (19 deuterated standards) • Silanisation of glassware not reported	Bisceglia et al. [36]

7 x ALC, 1 x antidepressant	Grab samples	Samples adjusted to pH2, and stored at 4 °C, extracted within 1 day. Samples filtered through 2.7 µm and 0.7 µm glass microfiber filters	SPE - Oasis HLB 60mg Condition: 2 mL MeOH, 2 mL H_2O (pH 7.5) Wash: None Elution: 4 mL MeOH	WWinf: 100 mL (pH 7.5); 200 x WWeff: 100 mL (pH 7.5); 200 x	Extract evaporated at 40 °C. Reconstituted in 500 µL of LC mobile phase A	WWinf[a, d]: AMP, 86; MAMP, 87; MDMA, 84; MDA, 76; MDEA, 90; nor-EPH, 22; EPH, 81; VENL, 125 WWeff[a, d]: AMP, 86; MAMP, 75; MDMA, 77; MDA, 74; MDEA, 84; nor-EPH, 55; EPH, 90; VENL, 122	0.2 µm PTFE filters	Chiral-UPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL[d]) AMP, 4.4; MAMP, 2.9; MDMA, 2.4; MDA, 9.7; MDEA, 2.2; nor-EPH, 11.3; EPH, 3.1; VENL, 5.0 (MDL[d]) AMP, 0.9; MAMP, 0.9; MDMA, 0.9; MDA, 2.0; MDEA, 0.6; nor-EPH, 3.4; EPH, 1.3; VENL, 1.6 WWeff: (MQL[d]) AMP, 4.4; MAMP, 3.4; MDMA, 2.6; MDA, 10.1; MDEA, 2.4; nor-EPH, 4.6; EPH, 2.8; VENL, 5.1 (MDL[d]) AMP, 0.9; MAMP, 1.0; MDMA, 1.0; MDA, 2.0; MDEA, 0.6; nor-EPH, 1.4; EPH, 1.1; VENL, 1.7	• First methodology for the chiral analysis of drugs of abuse in the environment • Matrix effects evaluated: Win, -43.9 - 56.3 • Internal standard quantification (5 deuterated standards) • Glassware silanised	Kasprzyk-Hordern et al. [37]
6 x opiates, 3 x ALC, 2 x cocainics, 1 x cannabinoid	24 h composite samples - flow dependant - at WWTP. Grab samples from RW	Samples adjusted to pH 2 and filtered through glass microfibre filters. Samples stored at 4 °C and extracted within 2 days	N/A - large volume direct injection	WWinf, WWeff, RW, LW: 1 mL (pH 2) (transferred to HPLC vial)	N/A - large volume direct injection	N/A - large volume direct injection	None reported	RP-HPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) COC, 20; BE, 20; AMP, 20; MAMP, 20; MDMA, 20; MOR, 20; COD, 20; 6ACM, 20; ACOD, 20; EDDP, 20; MET, 20; THC-COOH, 100	• Large volume direct injection • Internal standard quantification (12 deuterated standards) • Silanisation of glassware not reported	Berset et al. [38]
5 x opiates, 4 x ALC, 3 x cocainics, 2 x cannabinoids	Grab samples	Samples filtered through 1.6 µm glass microfiber filters. Samples stored at -20 °C and extracted within 7 days.	SPE - Oasis HLB 200 mg Condition: 5 mL MeOH, 5 mL H_2O Wash: 10 mL H_2O Elution: 6 mL MeOH	SW: 250 mL; 250 x	Extract evaporated at 45 °C. Reconstituted in 1000 µL H_2O:MeOH (75:25, v/v)	SW[a]: COC, 121±12; BE, 100±9; EME, 96±10; AMP, 96±10; MAMP, 113±11; MDMA, 125±8; MDA, 105±10; MOR, 75±10; HER, 100±12; 6ACM, 85±14; COD, 113±8;MET, 100±9; THC, 60±11; THC-COOH, 67±13	0.22 µm PTFE filter	RP-HPLC-ESI(+)-QqQ (2 SRM)	SW: (MQL) COC, 0.06; BE, 0.15; EME, 1.37; AMP, 0.40; MAMP, 0.75; MDMA, 0.35; MDA, 1.37; MOR, 0.13; HER, 0.17; 6ACM, 0.30; COD, 0.03; MET, 0.03; THC, 4.07; THC-COOH, 5.13 (MDL) COC, 0.02; BE, 0.05; EME, 0.41; AMP, 0.12; MAMP, 0.22; MDMA, 0.10; MDA, 0.41; MOR, 0.04; HER, 0.05; 6ACM, 0.09; COD, 0.01; MET, 0.01; THC, 1.22; THC-	• Comprehensive SPE sorbent, sample volume and sample pH investigation • Second injection monitoring fewer transitions to record two transitions for selected analytes • Matrix effects evaluated:	Vazquez-Roig et al. [8]

									COOH, 1.54	WWinf: SW, 0 - 20.2 % • Internal standard quantification (6 deuterated standards) • Silanisation of glassware not reported	
2 x cocainics, 4 x ALC	24 h composite samples	Samples stored at 4 °C and extracted within 2 days. Samples filtered through 1.5 µm glass microfiber filters	SPE - Oasis MCX 150 mg Condition: 6 mL MeOH, 10 mL H_2O (pH 3.0) Wash: 3 mL H_2O:5 % MeOH Elution: 2 x 3 mL MeOH:5 % NH_4OH	WWinf: 100 mL (pH 3.0); 250 x WWeff: 200 mL (pH 3.0); 500 x	Extract evaporated to almost dryness using a vacuum centrifuge apparatus. Reconstituted in H_2O:MeOH (1:1) to a final volume of 400 µL	Not reported	None reported	RP-HPLC-ESI(+)-ITMS (1 SRM)	WWInf: (MQL) COC, 20; BE, 20; AMP, 3; MAMP, 7; MDA, 22; MDMA, 9 (MDL) COC, 6; BE, 6; AMP, 1; MAMP, 2; MDA, 7; MDMA. 3	• Filtered particulates analysed • Internal standard quantification (6 deuterated standards) • Silanisation of glassware not reported	Metcalfe et al. [39]
2 x cocainics, 2 x ALC, 1 x opiates	24 h composite samples - flow weighted	Samples stored at 4 °C and extracted within 1 day. Samples filtered through 1.0 µm glass microfiber filters	SPE - Oasis HLB 500 mg Condition: 2 x 5 mL MeOH, 2 x 5 mL H_2O Wash: 5 mL H_2O Elution: 10 mL MeOH	WWinf: 250 mL; 500 x WWeff: 500 mL; 1000 x	Extracts evaporated (temperature not reported). Reconstituted in 500 µL MeOH	WWinf[b]: AMP, 74; COC, BE, MDMA, BUP 84-89	None reported	RP-HPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) COC, 1.2;, BE, 2.5; AMP, 12.8; MDMA, 3.0; BUP, 11.9 Wweff: (MQL) COC, 0.1; BE, 0.3; AMP, 2.6; MDMA, 0.5; BUP, 2.0	• Internal standard quantification (5 deuterated standards • Silanisation of glassware not reported	Karolak et al. [22]
1 x cocainic, 2 x ALC	24 h composite samples - flow dependant - and grab samples at	Samples stored at -20 °C. Samples filtered through 1.6 µm glass microfi	SPE - UCT XRDAH 500 mg Condition: N.R. Wash: 6 mL acetate buffer (pH 5.7), 2 mL 0.1 M acetic acid,	WWinf: 300 mL (pH 4.5 - 5); 1500 x	Extract evaporated (temperature not reported). Reconstituted in 20 µL	Water[f]: BE, 53.7-61.2; MAMP, 80.5-85.7, MDMA, 86.5-92.0	None reported	RP-HPLC-ESI(+)-QLIT (3 SRM)	Water: (MQL) BE, 10; MAMP, 2; MDMA, 2	• Relative recovery: Water, 98 - 102 % • Internal standard quantification (3 deuterated standards) • Silanisation of glassware not	Irvine et al. [40]

	WWTP	ber filters	6 mL MeOH Elution: 96 % dichloromethane:i-propanol (80:20) / 4 % ammonia (Volume not reported)		MeOH and mixed with 180 µL of 0.1 % HCOOH :H_2O					reported	
8 x cocainics, 10 x ALC, 20 x opiates, 6 x antidepressants, 7 x benzodiazepines, 3 x dissociative anesthetics, 2 x lysergics, 5 x urine markers, 2 x piperazines, 3 x other	Grab samples	Samples adjusted to pH1.8, and stored at 2 °C, extracted within 20 hrs. Samples filtered through 2.7 µm and 0.7 µm glass microfiber filters	SPE – Oasis MCX 60 mg Condition: 2 mL MeOH, 2 mL 2% HCOOH/H_2O (pH 2) Wash: 2 mL 2% HCOOH/H2O (pH 2), 2 mL 0.6% HCOOH/MeOH (pH 2) Elute: 3 mL 7% NH4OH/MeOH	WWinf: 100 mL (pH 1.8); 200 x	Extract evaporated at 40 °C. Reconstituted in 500 µL 0.3 % CH_3COOH, 5 % MeOH/ H_2O (v/v)	WWinf[b]: COC, 91±2; BE, 103±19; nor-BE, 93±4; nor-COC, 88±5; COE, 88±2; AME, 74±7; ECG, 77±5; AMP, 10±17; MAMP, 76±2; MCAT, 33±3; BZP, 101±18; TFMPP, 79±9; MDA, 90±3; MDMA, 86±3; MDEA, 86±6; MBDB, 88±5; BDB, 89±5; MES, 85±18; LSD, 80±2; O-H-LSD, 78±5; CAFF, 36±17; PARAX, 15±26; NIC, 76±9; COT, 37±2; HER, 3±0; 6ACM, 139±16; COD, 103±4; nor-COD, 91±3; OXYC, 57±3; OXYM, 68±6; MOR, 75±6; nor-MOR, 92±6; DICOD, 96±7; BUP, 77±5; nor-BUP, 85±4; MET, 97±6; EDDP, 38±4; EMDP, 55±3; FENT, 96±7; nor-FENT, 85±2; PRO, 90±4; nor-PRO, 99±9; TRAM, 69±7; nor-TRAM, 101±13; TEM, 15±8; DIAZ, 105±13; nor-DIAZ, 71±4; NIT, 92±3; 7AMN, 36±8; OXAZ, 54±5; CHL, 86±4; DOS, 67±3; AMIT, 80±6; nor-TRIP, 64±8; FLU, 61±4; nor-FLU, 62±9; VENL, 91±15; PCP, 80±2; KET, 70±5; nor-KET, 67±7; MAQ, 98±5; SILD, 81±23; EPH, 73±13; nor-EPH, 95±3	0.2 µm Whatman PTFE filter	RP-UPLC-ESI(+)-QqQ (2 SRM)	WWinf: (MQL) COC, 0.7; BE, 0.7; nor-BE, 0.8; nor-COC, 0.8; COE, 0.9; AME, 14.2; ECG, 15.2; AMP, 5.1; MAMP, 0.6; MCAT, 9.7; BZP, 9.6; TFMPP, 0.7; MDA, 4.2; MDMA, 0.7; MDEA, 1.1; MBDB, 0.7; BDB, 2.0; MES, 52.9; LSD, 0.7; O-H-LSD, 0.9; CAFF, 119.0; PARAX, 83.3; NIC, 4.7; COT, 34.0; HER, 139.9; 6ACM, 2.6; COD, 3.7; nor-COD, 3.4; OXYC, 4.9; OXYM, 10.5; MOR, 64.8; nor-MOR, 32.6; DICOD, 5.1; BUP, 8.9; nor-BUP, 5.1; MET, 0.8; EDDP, 2.0; EMDP, 1.0; FENT, 0.8; nor-FENT, 1.2; PRO, 5.2; nor-PRO, 21.2; TRAM, 5.1; nor-TRAM, 7.4; TEM, 16.6; DIAZ, 6.0; nor-DIAZ, 4.9; NIT, 3.2; 7AMN, 12.9; OXAZ, 5.9; CHL, 3.1; DOS, 4.5; AMIT, 4.8; nor-TRIP, 4.9; FLU, 3.0; nor-FLU, 2.7; VENL, 3.5; PCP, 0.5; KET, 1.0; nor-KET, 5.0; MAQ, 3.1; SILD, 4.6; EPH, 5.6; nor-EPH, 9.5	• Relative recovery: WWinf: 48 - 226 %; WWeff, 44 - 129 %; RW, 47 - 205 % • Internal standard quantification (31 deuterated standards) • Silanised glassware	Baker et al. [4]

	WWeff: 100mL (pH 1.8); 200 x RW: 500 mL (pH 1.8); 1000 x	WWeff: Due to number of compounds, refer to manuscript RW: Due to number of compounds, refer to manuscript	WWeff: Due to number of compounds, refer to manuscript RW: Due to number of compounds, refer to manuscript

ALC: amphetamine-like-compounds, COC: cocaine, BE: benzoylecgonine, EME: ecgonine methyl ester, COE: cocaethylene, nor-COC: norcocaine, nor-BE: norbenzoylecgonine, AME: anhydroecgonine methyl ester, ECG: ecgonidine, AMP: amphetamine, MAMP: methamphetamine, MCAT: methcathinone COD: codeine, nor-COD: norcodeine, DICOD: dihydrocodeine, MOR: morphine, M3G: morphine-3-glucuronide, 6ACM: 6-acetylmorphine, MET: methadone, OXYC: oxycodone, OXYM: oxymorphone, BUP: buprenorphine, nor-BUP: norbuprenorphine TRAM: tramadol, nor-TRAM: nortramadol, DIAZ: diazepam, nor-DIAZ: nordiazepam, NIT, nitrazepam, CHL: chlordiazepoxide, DOS: dosulepin, nor-TRIP: nortriptyline, FLU: Fluoxetine, VENL: venlafaxine, OXAZ: oxazepam, TEM: temazepam, 7AMN: 7-aminonitrazepam, KET: Ketamine, nor-KET: norketamine, FENT: fentanyl, nor-FENT: norfentanyl, PRO: propoxyphene, nor-PRO: norpropoxyphene, BZP: benzylpiperazine, TFMPP: Trifluoromethylphenylpiperazine, MES: mescaline, MAQ: methaqualone, SILD: sildenafil, CAFF: caffeine, PARAX: paraxanthine, NIC: nicotine, COT: continine, CREA: creatinine, EPH: ephedrine, nor-EPH: norephedrine, HER: heroin, FLUN: flunitrazepam, HYDC: hydrocodone, AMIT: amitriptyline, WWinf: wastewater influent, WWeff: wastewater effluent, RW: river water, SW: surface water, DW: drinking water

[a] Absolute recovery calculation: matrix spiked before SPE, against standard solution

[b] Absolute recovery calculation: matrix spiked before SPE, against matrix spiked after SPE

[c] Absolute recovery calculation: Compounds spiked before SPE, and IS added during reconstitution

[d] Average recovery value of enantiomers

[e] Value extracted from graph, therefore should be considered an approximate value

[f] On column detection limit (ng, 20 µL injection)

[g] Filter membrane not reported

n.e = not estimated due to high concentration found in the "blank" sample

n.r. = not reported in manuscript

Table 2 – Influence of silanisation on the recovery of compounds during evaporation (n = 3)

Compound	Comparison of silanised and non-silanised SPE extract vials, recovery (%)			
	7 % (v/v) NH_4OH/MeOH		MeOH	
	non-silanised	silanised	non-silanised	silanised
Stimulants				
Cocaine	64 ± 2	98 ± 4	87 ± 3	95 ± 5
Benzoylecgonine	101 ± 4	97 ± 7	95 ± 1	95 ± 3
Ecgonine methyl ester	37 ± 2	72 ± 4	76 ± 4	100 ± 3
Norbenzoylecgonine	96 ± 2	94 ± 4	96 ± 1	81 ± 4
Norcocaine	73 ± 1	99 ± 7	86 ± 1	95 ± 4
Cocaethylene	70 ± 3	95 ± 5	90 ± 0	93 ± 2
Anhydroecgonine methyl ester	28 ± 3	75 ± 1	32 ± 4	78 ± 3
Ecgonidine	114 ± 2	67 ± 10	117 ± 3	44 ± 1
Amphetamine	16 ± 3	88 ± 4	22 ± 2	67 ± 3
Methamphetamine	23 ± 4	89 ± 1	30 ± 3	81 ± 2
Methcathinone	16 ± 3	67 ± 4	20 ± 1	33 ± 2
BZP	37 ± 2	86 ± 2	55 ± 4	60 ± 1
TFMPP	37 ± 5	90 ± 3	54 ± 1	87 ± 3
Hallucinogens				
MDA	41 ± 3	99 ± 5	76 ± 1	83 ± 3
MDMA	40 ± 5	96 ± 6	75 ± 0	84 ± 3
MDEA	35 ± 4	97 ± 5	74 ± 3	81 ± 4
MBDB	27 ± 4	93 ± 2	64 ± 1	88 ± 2
BDB	27 ± 3	92 ± 5	58 ± 1	79 ± 3
Mescaline	76 ± 2	96 ± 9	79 ± 2	94 ± 6
LSD	91 ± 0	93 ± 3	87 ± 2	93 ± 3
O-H-LSD	83 ± 4	96 ± 7	80 ± 1	96 ± 3
Human indicators				
Caffeine	36 ± 2	99 ± 6	36 ± 2	90 ± 3
1,7-dimethylxanthine	117 ± 5	111 ± 10	106 ± 3	91 ± 4
Nicotine	11 ± 2	50 ± 13	10 ± 1	48 ± 3
Continine	44 ± 2	89 ± 12	52 ± 3	53 ± 0
Creatinine	83 ± 4	40 ± 11	106 ± 4	3 ± 0
Opioids and morphine derivatives				
Heroin	71 ± 7	68 ± 4	89 ± 3	85 ± 4
6-acetylmorphine	107 ± 5	118 ± 8	91 ± 2	91 ± 3
Codeine	95 ± 3	99 ± 9	94 ± 1	73 ± 3
Norcodeine	94 ± 8	97 ± 6	92 ± 1	68 ± 2
Oxycodone	75 ± 3	82 ± 5	81 ± 2	67 ± 2
Oxymorphone	101 ± 5	83 ± 9	102 ± 0	46 ± 1
Morphine	110 ± 5	102 ± 12	106 ± 1	58 ± 1
Normorphine	104 ± 4	92 ± 10	97 ± 2	57 ± 1
Dihydrocodeine	99 ± 1	100 ± 8	97 ± 2	77 ± 2
Buprenorphine	81 ± 2	89 ± 1	78 ± 0	81 ± 3
Norbuprenorphine	97 ± 2	95 ± 4	89 ± 1	88 ± 4
Methadone	50 ± 2	90 ± 4	67 ± 1	79 ± 5
EDDP	16 ± 2	89 ± 2	60 ± 3	85 ± 3
EMDP	8 ± 2	30 ± 1	1 ± 0	64 ± 6
Fentanyl	93 ± 2	98 ± 4	90 ± 1	90 ± 1
Norfentanyl	96 ± 4	98 ± 6	90 ± 1	87 ± 2
Propoxyphene	45 ± 4	96 ± 5	64 ± 3	92 ± 2

Norpropoxyphene	676	±	7	638	±	51	119	±	5	246	±	14
Tramadol	37	±	3	89	±	5	72	±	2	83	±	3
Nortramadol	87	±	7	98	±	13	81	±	2	89	±	1
Benzodiazepines												
Temazepam	90	±	2	89	±	3	86	±	3	90	±	0
Diazepam	93	±	3	88	±	5	90	±	1	97	±	4
Nordiazepam	91	±	5	85	±	2	84	±	2	76	±	3
Nitrazepam	73	±	3	66	±	3	80	±	6	69	±	6
7-aminonitrazepam	78	±	2	94	±	6	58	±	5	82	±	7
Oxazepam	91	±	3	87	±	4	86	±	2	101	±	4
Chlordiazepoxide	94	±	3	107	±	7	86	±	3	87	±	1
Antidepressants												
Dosulepin	82	±	1	90	±	6	47	±	2	47	±	4
Amitriptyline	58	±	3	90	±	3	45	±	0	58	±	3
Nortriptyline	83	±	2	79	±	4	39	±	2	47	±	5
Fluoxetine	71	±	2	82	±	7	19	±	2	25	±	4
Norfluoxetine	72	±	5	74	±	5	19	±	1	27	±	4
Venlafaxine	61	±	4	100	±	5	84	±	0	95	±	6
Dissociative anaesthetics												
Phencyclidine	20	±	4	86	±	6	26	±	2	95	±	3
Ketamine	20	±	3	84	±	3	22	±	2	88	±	2
Norketamine	20	±	3	74	±	3	20	±	3	63	±	3
Other												
Methaqualone	33	±	2	83	±	4	21	±	2	94	±	4
Sildenafil	86	±	4	82	±	9	40	±	2	56	±	5
Drug precursors												
Ephedrine	32	±	5	100	±	7	59	±	2	59	±	5
Norephedrine	42	±	5	114	±	22	62	±	5	33	±	2

Table 3 – Stability of analytes (n = 2) in WWTP influent stored over a 72 hour period in raw (unfiltered) wastewater

Compound	Difference (%) to time-point 0 ± SD (%)																	
	Raw (unfiltered) wastewater, pH 7.4, stored at 2 °C									Raw (unfiltered) wastewater, pH 7.4, stored at 19 °C								
	12 hours			24 hours			72 hours			12 hours			24 hours			72 hours		
Stimulants																		
Cocaine	-5.1	±	2.8	-11.8	±	3.6	-8.2	±	3.9	-7.7	±	1.0	-12.3	±	2.8	-23.1	±	2.1
Benzoylecgonine	2.5	±	1.4	0.5	±	0.2	0.5	±	1.4	5.5	±	3.0	7.4	±	5.4	11.7	±	6.4
Norbenzoylecgonine	-2.9	±	1.8	-7.5	±	2.9	-6.0	±	0.8	3.5	±	6.1	0.6	±	4.0	-4.4	±	0.0
Norcocaine	-14.7	±	2.6	-19.5	±	4.3	-14.4	±	1.0	-9.9	±	5.4	-20.6	±	1.7	-31.5	±	1.4
Cocaethylene	-4.0	±	6.2	-5.4	±	4.3	-9.4	±	7.6	-6.8	±	6.1	-8.6	±	9.1	-19.0	±	2.8
Anhydroecgonine methyl ester	12.0	±	2.6	7.2	±	1.1	12.6	±	5.3	19.8	±	0.1	26.5	±	11.7	52.7	±	5.3
Ecgonidine	-0.8	±	16.7	5.4	±	3.9	24.0	±	6.2	30.8	±	1.1	20.4	±	7.6	83.0	±	7.4
Amphetamine	6.9	±	2.6	26.0	±	1.8	36.8	±	0.8	46.8	±	5.6	73.8	±	0.4	88.2	±	13.0
Methamphetamine	-10.0	±	16.3	-14.4	±	8.5	-6.0	±	9.7	8.1	±	5.9	5.5	±	1.9	12.0	±	7.9
Methcathinone	-50.7	±	12.4	-57.4	±	2.7	-60.3	±	3.3	-56.5	±	0.7	-66.4	±	1.8	-77.8	±	6.0
BZP	70.0	±	15.5	78.3	±	12.9	80.7	±	7.4	55.6	±	21.7	78.4	±	8.4	115.0	±	25.3
TFMPP	25.6	±	8.2	28.3	±	5.6	34.5	±	4.8	23.5	±	23.9	39.1	±	6.4	36.9	±	2.0
Hallucinogens																		
MDA	0.0	±	2.2	-2.0	±	3.7	-3.5	±	9.7	3.4	±	5.4	-2.2	±	1.2	-10.5	±	1.2
MDMA	-3.8	±	1.8	-6.6	±	0.3	-3.1	±	0.6	1.4	±	1.6	2.8	±	1.6	-0.4	±	0.7
MDEA	4.5	±	0.0	-1.6	±	1.9	0.4	±	2.5	-1.5	±	0.5	2.4	±	4.1	-12.1	±	4.0
MBDB	-17.7	±	2.7	-17.4	±	0.4	-14.3	±	0.8	-8.6	±	3.1	-10.2	±	2.6	-21.7	±	7.1
BDB	-35.0	±	18.7	-38.3	±	8.8	-33.0	±	8.6	-20.5	±	7.3	-19.5	±	0.4	-31.2	±	9.3
Mescaline	-8.1	±	8.9	-12.9	±	8.9	-10.2	±	9.1	-7.1	±	0.9	-17.1	±	9.7	-26.7	±	8.1
LSD	-6.1	±	2.4	-13.6	±	0.4	-9.9	±	1.6	-3.5	±	1.5	-6.8	±	1.3	-20.4	±	1.7
O-H-LSD	5.3	±	8.6	1.7	±	6.1	-2.8	±	4.9	-4.7	±	17.5	-9.1	±	14.0	-13.6	±	0.8
Human indicators																		
Caffeine	6.3	±	4.1	2.7	±	17.9	30.3	±	9.1	8.4	±	21.3	22.9	±	25.8	45.0	±	20.0
1,7-dimethylxanthine	-25.0	±	15.5	-26.0	±	0.8	-19.9	±	11.9	-15.3	±	16.8	-25.2	±	4.8	-40.8	±	17.4
Nicotine	0.2	±	2.2	1.0	±	1.3	-2.0	±	1.6	3.8	±	2.3	0.9	±	1.4	1.0	±	1.5
Contnine	23.9	±	27.8	4.4	±	3.5	37.1	±	5.1	56.5	±	35.0	61.4	±	67.1	163.2	±	21.0
Opioids and morphine derivatives																		
Heroin	-66.2	±	3.7	-82.3	±	0.6	-97.5	±	0.1	-79.4	±	0.6	-95.5	±	0.3	-99.9	±	0.1
6-acetylmorphine	-26.4	±	3.3	-32.5	±	0.5	-54.6	±	0.3	-12.0	±	5.1	-41.5	±	2.1	-76.6	±	0.4
Codeine	6.9	±	5.6	8.4	±	5.2	15.2	±	3.0	12.4	±	3.6	11.7	±	2.9	9.5	±	2.1
Norcodeine	-1.6	±	0.7	-3.9	±	5.2	4.4	±	2.1	4.5	±	3.0	-1.4	±	2.1	-9.6	±	2.5
Oxycodone	8.3	±	9.6	1.7	±	4.9	6.8	±	6.4	9.6	±	0.4	10.0	±	0.4	5.8	±	2.1
Oxymorphone	22.2	±	4.5	21.3	±	9.6	26.4	±	10.3	31.0	±	0.3	31.2	±	3.6	42.9	±	2.4
Morphine	46.2	±	0.0	62.4	±	4.8	83.5	±	0.0	48.9	±	0.2	74.6	±	11.8	89.1	±	4.5
Morphine-3β-glucuronide	-80.5	±	3.0	-90.3	±	0.2	-98.9	±	0.2	-84.7	±	0.7	-98.3	±	0.3	<MQL		
Normorphine	10.6	±	7.8	14.4	±	0.1	12.4	±	0.5	4.3	±	3.9	12.7	±	0.0	3.1	±	0.3
Dihydrocodeine	-5.9	±	8.3	-6.6	±	5.1	-8.3	±	3.3	-6.0	±	7.0	-1.2	±	2.7	-1.2	±	10.6
Buprenorphine	4.3	±	2.3	-4.7	±	6.7	-3.0	±	3.4	-5.9	±	4.3	-1.9	±	5.1	-17.4	±	1.3

Norbuprenorphine	6.5	±	1.5	3.6	±	5.4	0.7	±	0.5	-3.7	±	2.3	-0.1	±	10.3	-5.5	±	6.6
Methadone	-5.8	±	5.5	-14.4	±	7.6	-10.9	±	9.3	-6.7	±	1.0	-4.2	±	4.1	-23.4	±	1.6
EDDP	-14.8	±	6.5	-15.3	±	5.3	-17.1	±	9.9	-12.8	±	2.1	-17.7	±	1.9	-71.9	±	2.5
EMDP	-9.3	±	0.2	-8.4	±	1.6	-16.6	±	3.4	-17.7	±	10.7	-7.2	±	0.0	-41.3	±	3.7
Fentanyl	-10.8	±	1.9	-14.0	±	1.7	-16.2	±	0.8	-8.3	±	11.1	-14.0	±	1.8	-61.6	±	0.8
Norfentanyl	1.9	±	2.1	-8.8	±	4.1	-18.6	±	6.1	-13.5	±	9.1	-19.9	±	0.8	-23.1	±	1.8
Propoxyphene	-1.1	±	3.0	-10.5	±	10.1	0.0	±	14.7	-1.8	±	4.1	10.1	±	9.2	24.6	±	3.5
Norpropoxyphene	48.7	±	4.1	72.6	±	22.2	115.7	±	29.5	64.0	±	9.0	114.6	±	13.1	123.3	±	59.1
Tramadol	-16.2	±	5.9	-18.0	±	4.8	-17.6	±	3.0	-11.0	±	3.8	-19.5	±	6.1	-32.7	±	0.6
Nortramadol	-49.8	±	12.0	-60.8	±	3.8	-56.4	±	10.8	-44.2	±	4.2	-61.1	±	6.6	-63.0	±	0.8
Benzodiazepines																		
Temazepam	-5.7	±	2.7	1.8	±	1.8	1.7	±	0.8	19.4	±	21.0	17.3	±	2.7	-1.1	±	14.2
Diazepam	-13.2	±	12.4	-15.3	±	15.6	-15.1	±	16.1	-3.0	±	8.3	-9.3	±	18.4	-17.1	±	13.8
Nordiazepam	15.2	±	10.1	6.3	±	6.9	5.7	±	2.7	15.6	±	1.2	21.4	±	21.7	49.7	±	5.1
Nitrazepam	-29.8	±	10.0	-57.1	±	3.9	-81.5	±	1.3	-61.9	±	1.5	-87.4	±	3.5	-99.8	±	0.1
7-aminonitrazepam	6.4	±	10.0	9.3	±	2.5	44.4	±	12.7	29.8	±	14.2	42.1	±	0.7	40.5	±	3.3
Oxazepam	-6.2	±	8.4	-11.4	±	10.0	-1.3	±	8.0	2.4	±	12.3	7.9	±	2.9	1.3	±	15.4
Chlordiazepoxide	1.7	±	18.9	4.5	±	13.1	-23.6	±	4.5	-14.4	±	6.2	-12.7	±	31.2	12.8	±	3.4
Antidepressants																		
Dosulepin	-31.5	±	1.5	-50.0	±	5.9	-40.8	±	1.8	-7.4	±	4.3	-16.2	±	20.1	-72.3	±	6.5
Amitriptyline	-18.5	±	1.3	-37.9	±	11.3	-22.9	±	2.0	10.1	±	6.6	6.8	±	33.4	-61.2	±	7.2
Nortriptyline	-34.5	±	6.2	-58.8	±	9.3	-42.0	±	9.4	-7.8	±	11.6	-11.7	±	44.0	-78.9	±	8.6
Fluoxetine	-5.5	±	1.5	-37.0	±	1.1	-27.7	±	0.5	8.2	±	8.8	1.0	±	20.3	-54.8	±	1.6
Norfluoxetine	-13.8	±	17.0	-49.4	±	3.2	-46.2	±	10.1	1.9	±	11.4	4.8	±	57.4	-56.1	±	10.2
Venlafaxine	-5.7	±	1.5	-16.6	±	8.1	-24.9	±	2.9	-20.5	±	15.9	-29.2	±	3.6	-43.6	±	3.8
Dissociative anaesthetics																		
Phencyclidine	-1.9	±	0.9	-3.1	±	1.9	-1.2	±	1.1	-3.2	±	1.6	0.6	±	7.0	-25.2	±	3.5
Ketamine	-1.9	±	1.1	-4.1	±	1.4	-1.4	±	1.0	-0.8	±	1.2	1.7	±	3.7	0.5	±	5.0
Norketamine	-6.9	±	0.9	-10.9	±	3.3	-5.1	±	0.0	-2.7	±	2.7	-5.2	±	2.5	2.6	±	5.2
Other																		
Methaqualone	-11.9	±	12.3	-17.1	±	17.9	-2.2	±	11.4	-2.7	±	6.9	1.1	±	17.8	-10.1	±	1.1
Sildenafil	3.4	±	8.3	5.8	±	18.4	27.9	±	17.0	11.5	±	19.0	7.1	±	28.5	-53.4	±	13.7
Drug precursors																		
Ephedrine	-38.1	±	16.6	-25.6	±	16.6	-32.8	±	20.5	-40.4	±	4.3	-49.2	±	1.9	-56.6	±	0.1
Norephedrine	-59.3	±	10.1	-63.6	±	6.3	-79.9	±	3.7	-65.1	±	3.0	-73.3	±	1.9	-83.6	±	2.0

Table 4 – Stability of analytes (n = 2) in WWTP influent stored over a 72 hour period in filtered wastewater at pH 1.8

Compound	Difference (%) to time-point 0 ± SD (%)											
	Filtered wastewater, pH 1.8, stored at 2 °C						Filtered wastewater, pH 1.8, stored at 19 °C					
	12 hours		24 hours		72 hours		12 hours		24 hours		72 hours	
Stimulants												
Cocaine	-0.6	± 6.5	0.7	± 3.5	-5.0	± 4.2	-7.9	± 1.0	-9.0	± 1.0	-6.4	± 2.7
Benzoylecgonine	0.9	± 3.6	-0.4	± 1.9	-9.4	± 3.5	-4.6	± 5.0	-5.1	± 4.3	-6.2	± 0.1
Norbenzoylecgonine	2.5	± 2.5	0.0	± 0.4	-11.4	± 3.3	-3.0	± 5.1	-2.6	± 1.8	-4.1	± 2.3
Norcocaine	-1.3	± 3.5	-3.7	± 1.2	-7.9	± 3.5	-0.4	± 3.3	-7.6	± 1.0	-7.7	± 5.8
Cocaethylene	0.0	± 0.5	-0.5	± 5.5	-6.6	± 9.1	-3.9	± 2.7	-3.1	± 3.1	-3.9	± 2.6
Anhydroecgonine methyl ester	-2.6	± 7.6	-2.3	± 6.6	-7.2	± 4.8	-10.5	± 4.8	-11.1	± 1.0	-14.5	± 2.9
Ecgonidine	1.9	± 6.8	11.8	± 4.5	2.3	± 15.0	-8.7	± 9.9	-5.1	± 29.8	-10.0	± 14.1
Amphetamine	-6.4	± 7.6	-7.6	± 7.3	-8.7	± 4.3	-7.6	± 2.8	-10.6	± 3.4	-11.3	± 3.0
Methamphetamine	-7.3	± 11.8	-8.1	± 15.6	-14.3	± 16.2	-8.1	± 5.8	-3.9	± 1.0	-8.2	± 0.9
Methcathinone	5.9	± 20.1	4.8	± 13.5	0.8	± 23.9	-5.5	± 14.1	-4.9	± 11.3	1.0	± 9.2
BZP	2.0	± 5.0	4.9	± 8.2	8.4	± 11.0	-3.7	± 1.9	-1.0	± 3.1	1.4	± 1.0
TFMPP	0.9	± 1.5	3.9	± 6.7	3.7	± 4.8	-0.9	± 9.5	-0.4	± 9.1	-1.2	± 7.7
Hallucinogens												
MDA	2.6	± 2.1	10.0	± 3.1	-0.5	± 4.0	-5.8	± 3.6	-3.1	± 1.3	-4.1	± 0.3
MDMA	-6.0	± 4.4	-7.5	± 3.1	-11.4	± 5.0	-2.7	± 4.8	-5.0	± 2.7	-3.2	± 0.4
MDEA	-1.4	± 4.1	-1.2	± 5.9	-7.3	± 7.1	-3.3	± 8.4	-6.8	± 1.6	-7.5	± 2.1
MBDB	-0.8	± 5.4	-2.8	± 6.1	-9.6	± 4.1	-5.4	± 9.0	-2.2	± 7.4	-4.5	± 5.6
BDB	-3.8	± 7.0	-13.1	± 4.7	-21.8	± 2.2	-2.5	± 11.6	2.6	± 0.8	-4.5	± 2.0
Mescaline	-1.8	± 1.8	-9.3	± 0.5	-14.0	± 7.7	-1.1	± 1.0	-3.9	± 1.3	-7.3	± 1.4
LSD	-2.7	± 0.5	-3.5	± 3.7	-16.0	± 0.5	-6.1	± 11.5	-3.9	± 2.7	-6.6	± 3.5
O-H-LSD	1.6	± 9.5	1.8	± 7.1	-3.8	± 16.3	-4.3	± 12.3	-4.8	± 5.1	-2.6	± 3.3
Human indicators												
Caffeine	0.0	± 2.4	-11.9	± 9.5	-15.2	± 5.1	-8.0	± 3.6	-7.7	± 15.6	-3.1	± 10.7
1,7-dimethylxanthine	4.2	± 9.2	0.7	± 13.9	1.9	± 19.4	-11.4	± 3.2	-19.9	± 4.5	-15.2	± 5.5
Nicotine	-1.4	± 0.5	-7.8	± 4.4	-7.5	± 0.4	-9.6	± 3.8	-15.2	± 2.2	-17.2	± 1.5
Continine	3.0	± 16.7	-7.2	± 16.6	-13.5	± 30.1	-7.1	± 13.3	-3.5	± 31.2	4.7	± 29.8
Opioids and morphine derivatives												
Heroin	2.1	± 4.3	3.9	± 3.9	-7.5	± 3.0	-3.3	± 3.5	-6.5	± 1.6	-13.2	± 1.6
6-acetylmorphine	-0.6	± 1.5	-5.0	± 2.9	-9.2	± 6.6	-4.7	± 0.1	-5.9	± 0.5	-10.2	± 2.1
Codeine	2.1	± 2.9	-1.5	± 0.4	-4.3	± 3.2	-1.3	± 0.7	-1.3	± 1.8	-0.6	± 3.3
Norcodeine	1.3	± 1.6	-2.1	± 3.6	-7.0	± 3.8	-2.1	± 0.4	-2.9	± 0.5	-4.3	± 0.0
Oxycodone	-3.6	± 0.3	-3.1	± 2.0	-9.0	± 1.4	-2.1	± 4.0	-3.4	± 2.2	-8.7	± 2.9
Oxymorphone	-4.2	± 8.8	-5.5	± 3.7	-11.9	± 4.6	-4.6	± 8.4	-5.0	± 5.1	-9.7	± 6.5
Morphine	2.4	± 8.4	-1.4	± 3.9	-5.5	± 2.3	-8.2	± 10.4	-8.9	± 5.5	-9.3	± 0.7
Morphine-3β-glucuronide	3.1	± 18.4	6.7	± 3.1	13.4	± 18.5	-4.9	± 15.7	6.7	± 22.2	-5.2	± 1.0
Normorphine	5.3	± 3.1	1.6	± 9.0	-4.0	± 2.7	-2.1	± 13.2	-6.8	± 3.3	-4.5	± 1.4
Dihydrocodeine	0.5	± 3.6	-1.2	± 2.1	-7.1	± 4.1	-3.8	± 9.0	-4.5	± 8.8	-2.6	± 7.3
Buprenorphine	-0.2	± 1.6	-0.1	± 1.3	-6.9	± 2.7	-0.3	± 4.1	0.6	± 1.5	1.1	± 2.8

Norbuprenorphine	3.3	±	1.2	2.7	±	0.3	-4.4	±	1.2	-0.6	±	0.2	-1.7	±	0.0	-3.4	±	1.0
Methadone	-0.4	±	1.4	-3.1	±	0.8	-11.0	±	0.9	-6.4	±	4.6	-4.4	±	1.2	-6.3	±	6.0
EDDP	-1.5	±	2.3	4.1	±	9.7	-7.6	±	8.4	-3.0	±	7.1	-2.5	±	6.0	-1.2	±	11.6
EMDP	-5.4	±	11.2	-17.7	±	11.6	-28.6	±	4.7	-12.5	±	0.6	-17.2	±	4.9	-23.7	±	0.8
Fentanyl	2.8	±	2.2	-2.4	±	0.6	-7.5	±	4.3	-0.6	±	2.3	0.8	±	0.9	1.7	±	0.1
Norfentanyl	-4.0	±	2.1	-7.0	±	3.0	-9.8	±	0.4	-2.8	±	2.5	-2.2	±	5.8	-5.4	±	7.4
Propoxyphene	-3.3	±	10.4	1.5	±	6.1	-12.7	±	6.4	-4.2	±	0.4	-0.4	±	1.7	-4.0	±	1.8
Norpropoxyphene	-3.5	±	5.7	-10.3	±	12.4	-21.2	±	0.8	-5.3	±	14.1	-10.2	±	1.1	-14.6	±	4.1
Tramadol	2.6	±	12.4	-4.1	±	9.6	-7.1	±	10.0	1.8	±	2.7	1.4	±	2.3	2.3	±	1.7
Nortramadol	-9.5	±	3.1	-16.7	±	0.9	-22.7	±	0.2	11.0	±	9.9	1.1	±	3.0	-0.6	±	15.0
Benzodiazepines																		
Temazepam	-2.7	±	9.9	-18.0	±	0.4	-23.4	±	5.1	-22.3	±	2.9	-23.0	±	0.4	-62.7	±	0.1
Diazepam	-3.1	±	14.7	-6.2	±	14.9	-23.3	±	12.9	-2.7	±	1.6	-7.4	±	2.0	-21.1	±	3.5
Nordiazepam	0.8	±	17.6	2.7	±	16.1	-4.0	±	14.7	0.9	±	7.8	1.8	±	15.6	-22.0	±	0.3
Nitrazepam	-16.7	±	9.4	-24.9	±	4.9	-35.1	±	7.7	-10.5	±	10.4	-25.2	±	9.5	-41.8	±	1.9
7-aminonitrazepam	5.9	±	10.8	11.0	±	10.0	18.9	±	15.2	9.7	±	3.1	1.2	±	8.2	5.6	±	11.1
Oxazepam	-0.2	±	1.3	-1.4	±	7.3	-8.6	±	13.6	-8.2	±	0.6	-9.0	±	4.4	-29.4	±	1.1
Chlordiazepoxide	-3.8	±	17.7	-4.5	±	9.7	-14.1	±	13.7	9.7	±	10.4	4.2	±	15.2	-8.1	±	3.1
Antidepressants																		
Dosulepin	-7.6	±	16.4	-8.2	±	9.5	-25.4	±	9.4	1.1	±	0.2	4.6	±	6.3	-2.4	±	14.2
Amitriptyline	-5.4	±	15.1	-6.9	±	11.2	-23.6	±	8.7	-1.6	±	5.8	-2.6	±	8.1	-8.7	±	10.5
Nortriptyline	-11.9	±	18.1	-18.1	±	15.0	-32.6	±	13.6	-4.2	±	8.5	-5.9	±	8.7	-20.9	±	14.8
Fluoxetine	-1.4	±	6.3	-6.1	±	7.4	-15.1	±	6.9	-4.6	±	3.3	-3.5	±	1.8	-14.6	±	0.6
Norfluoxetine	-10.1	±	18.8	-13.0	±	16.3	-36.4	±	11.5	-2.8	±	1.5	-7.7	±	3.1	-32.6	±	4.6
Venlafaxine	-2.8	±	3.5	-8.0	±	3.1	-12.7	±	7.8	2.3	±	10.5	-0.7	±	6.7	3.9	±	6.4
Dissociative anaesthetics																		
Phencyclidine	-6.5	±	4.4	-4.8	±	0.4	-12.4	±	1.6	-3.2	±	6.3	-7.8	±	5.5	-2.3	±	9.2
Ketamine	2.2	±	1.7	0.7	±	1.1	-3.2	±	2.9	-3.9	±	0.7	-2.5	±	6.7	-3.0	±	6.1
Norketamine	3.8	±	2.4	2.2	±	0.6	-0.8	±	4.6	-4.7	±	2.7	-5.0	±	3.4	-3.1	±	0.8
Other																		
Methaqualone	-8.8	±	12.2	-13.7	±	6.2	-19.6	±	9.4	-6.6	±	0.8	-11.3	±	7.5	-17.9	±	2.5
Sildenafil	3.0	±	9.8	7.9	±	3.7	-2.1	±	1.1	-1.9	±	3.1	9.1	±	0.9	10.4	±	0.5
Drug precursors																		
Ephedrine	-3.9	±	2.4	-5.9	±	7.2	-0.4	±	10.6	-6.1	±	3.2	-3.1	±	8.0	1.7	±	11.2
Norephedrine	-4.4	±	9.9	2.6	±	8.2	-1.3	±	10.6	-2.0	±	1.3	4.0	±	6.6	4.5	±	9.5

Table 5 – Stability of analytes (n = 2) in WWTP influent stored over a 72 hour period in filtered wastewater at pH 7.4

Compound	Difference (%) to time-point 0 ± SD (%)																	
	Filtered wastewater, 7.4, stored at 2 °C									Filtered wastewater, pH 7.4, stored at 19 °C								
	12 hours			24 hours			72 hours			12 hours			24 hours			72 hours		
Stimulants																		
Cocaine	-4.9	±	0.5	-4.3	±	0.5	-9.6	±	2.4	-12.1	±	1.3	-13.6	±	0.7	-28.3	±	0.1
Benzoylecgonine	2.7	±	2.0	1.1	±	3.5	3.4	±	5.3	0.7	±	2.2	9.1	±	1.4	17.3	±	6.1
Norbenzoylecgonine	-3.1	±	1.1	-5.2	±	4.0	-1.9	±	6.4	-4.9	±	1.8	-1.4	±	0.2	-0.1	±	3.7
Norcocaine	-4.6	±	0.8	-3.7	±	0.4	-7.5	±	1.6	-7.8	±	5.5	-7.6	±	2.6	-9.2	±	2.4
Cocaethylene	-1.7	±	3.3	-4.3	±	6.2	-8.7	±	3.4	-9.4	±	3.5	-7.1	±	2.0	-15.5	±	1.9
Anhydroecgonine methyl ester	9.4	±	6.6	28.4	±	5.4	41.1	±	5.6	-0.9	±	2.2	22.4	±	3.4	45.3	±	3.8
Ecgonidine	3.0	±	3.4	15.1	±	15.8	27.1	±	21.6	6.0	±	4.9	38.0	±	5.9	43.3	±	0.3
Amphetamine	23.4	±	9.7	28.9	±	6.2	44.3	±	9.7	14.7	±	1.8	21.1	±	0.6	21.3	±	2.9
Methamphetamine	2.9	±	0.6	5.9	±	2.9	3.5	±	1.3	-2.7	±	9.9	3.7	±	7.9	-6.1	±	5.2
Methcathinone	-29.3	±	1.7	-34.6	±	3.1	-42.8	±	1.2	-18.0	±	8.9	-13.6	±	8.8	-42.1	±	10.5
BZP	25.1	±	9.9	49.3	±	3.9	60.2	±	5.0	8.8	±	5.1	20.8	±	3.1	17.8	±	5.8
TFMPP	10.0	±	3.4	7.9	±	10.9	-22.9	±	16.9	4.7	±	0.2	-32.4	±	3.0	-50.5	±	19.5
Hallucinogens																		
MDA	3.7	±	4.4	0.3	±	6.0	-0.4	±	5.3	2.2	±	8.6	-2.1	±	10.9	-9.2	±	0.7
MDMA	0.8	±	2.7	4.8	±	6.7	-5.9	±	4.8	-5.3	±	2.1	-5.5	±	0.4	-4.7	±	4.3
MDEA	-2.2	±	0.0	-4.8	±	0.9	-3.5	±	3.6	-4.0	±	6.2	-4.2	±	1.6	-6.1	±	4.1
MBDB	-6.2	±	1.3	-8.8	±	4.6	-9.4	±	0.9	-5.8	±	5.0	-5.3	±	6.4	-4.9	±	2.8
BDB	-11.9	±	3.0	-10.6	±	5.6	-14.5	±	6.4	-15.0	±	8.1	-13.1	±	5.0	-5.9	±	5.2
Mescaline	-5.5	±	1.7	-10.5	±	0.7	-20.1	±	3.2	-11.2	±	5.1	-10.1	±	7.7	-21.3	±	17.4
LSD	1.3	±	4.8	3.0	±	7.3	0.4	±	0.3	-6.6	±	1.4	-2.9	±	0.4	-4.1	±	2.3
O-H-LSD	7.0	±	1.6	13.4	±	1.7	42.4	±	24.1	-6.8	±	2.9	28.3	±	5.6	83.4	±	9.5
Human indicators																		
Caffeine	12.0	±	8.7	17.3	±	1.1	29.7	±	11.4	-3.4	±	1.2	22.9	±	4.8	47.7	±	8.0
1,7-dimethylxanthine	-6.6	±	9.4	-18.3	±	10.6	-8.9	±	22.2	-3.9	±	5.7	1.1	±	4.7	52.9	±	4.2
Nicotine	-3.9	±	0.9	-1.2	±	4.8	1.1	±	2.9	-2.7	±	4.2	-1.2	±	0.9	3.6	±	7.5
Contnine	31.7	±	24.2	12.6	±	12.0	70.9	±	68.7	-17.1	±	15.0	58.2	±	23.2	207.7	±	40.8
Opioids and morphine derivatives																		
Heroin	-21.4	±	3.7	-38.7	±	3.5	-68.4	±	0.5	-37.2	±	1.9	-63.4	±	0.9	-94.5	±	0.3
6-acetylmorphine	-1.8	±	2.0	-11.0	±	3.3	-11.3	±	0.3	-4.9	±	8.8	-7.9	±	3.3	-23.6	±	9.7
Codeine	13.1	±	4.8	15.9	±	7.1	23.8	±	2.6	9.3	±	3.4	16.1	±	1.8	18.4	±	1.9
Norcodeine	-0.4	±	1.4	-3.8	±	6.1	-2.3	±	1.1	-4.5	±	3.0	-4.5	±	2.1	-6.7	±	3.8
Oxycodone	10.0	±	9.6	12.8	±	0.6	5.2	±	1.3	-3.9	±	0.7	3.0	±	3.9	-3.1	±	4.2
Oxymorphone	18.2	±	8.9	20.3	±	8.7	13.7	±	2.6	4.3	±	0.4	10.8	±	2.3	19.3	±	3.2
Morphine	33.2	±	9.3	35.0	±	11.2	45.3	±	5.2	39.8	±	3.3	49.0	±	2.4	58.8	±	6.5
Morphine-3β-glucuronide	-51.0	±	3.2	-77.7	±	0.0	-93.7	±	0.1	-84.7	±	0.4	-96.1	±	0.3	-99.1	±	0.2
Normorphine	2.7	±	7.5	-0.7	±	11.9	-0.4	±	11.8	8.9	±	2.8	6.8	±	2.6	-0.1	±	5.7
Dihydrocodeine	2.2	±	0.4	1.1	±	1.4	1.0	±	1.6	3.3	±	0.5	9.4	±	1.3	9.3	±	1.6
Buprenorphine	3.4	±	4.5	2.8	±	0.7	2.1	±	1.9	-1.2	±	8.0	-3.1	±	2.0	-7.4	±	4.2

Norbuprenorphine	-0.4	±	4.9	4.2	±	0.5	16.9	±	2.5	-7.5	±	3.2	-3.3	±	1.1	10.0	±	6.4
Methadone	4.2	±	3.5	-3.4	±	6.9	-11.1	±	5.7	1.9	±	4.8	-4.7	±	1.1	-11.3	±	1.9
EDDP	-2.3	±	3.8	-13.2	±	4.3	-16.9	±	1.1	2.6	±	19.9	-15.5	±	0.8	-17.5	±	6.6
EMDP	-4.4	±	11.5	6.0	±	6.6	12.6	±	10.6	-7.8	±	0.1	9.7	±	6.1	33.0	±	36.5
Fentanyl	0.0	±	0.3	-1.8	±	0.3	-4.8	±	1.3	0.6	±	2.0	-3.6	±	0.4	-6.1	±	1.6
Norfentanyl	-8.5	±	4.5	1.6	±	4.4	9.7	±	15.4	-5.1	±	0.0	1.2	±	2.4	21.4	±	0.2
Propoxyphene	-4.3	±	3.4	-6.7	±	1.8	-7.9	±	1.1	-0.9	±	0.6	-15.4	±	2.1	-18.9	±	0.6
Norpropoxyphene	71.2	±	7.8	96.5	±	4.8	132.4	±	18.9	109.8	±	20.7	143.7	±	28.4	175.8	±	23.8
Tramadol	-6.0	±	3.3	-13.2	±	1.3	-16.6	±	2.1	-7.0	±	7.9	-13.5	±	1.2	-17.6	±	0.3
Nortramadol	-23.1	±	3.9	-40.8	±	2.3	-38.3	±	3.3	-16.2	±	5.4	-34.4	±	4.3	-37.0	±	4.6
Benzodiazepines																		
Temazepam	4.8	±	10.3	-0.9	±	13.5	-3.4	±	8.0	2.3	±	15.7	11.6	±	8.3	7.5	±	17.0
Diazepam	-23.4	±	12.9	-25.2	±	10.4	-27.7	±	7.2	-18.4	±	2.5	-36.7	±	2.9	-44.0	±	1.8
Nordiazepam	13.8	±	11.3	21.5	±	29.8	20.0	±	6.0	1.9	±	1.2	7.8	±	4.7	-1.2	±	7.3
Nitrazepam	-13.8	±	3.3	-21.8	±	9.5	-40.1	±	4.2	-57.4	±	2.1	-77.2	±	1.4	-97.2	±	0.2
7-aminonitrazepam	7.0	±	5.0	-8.1	±	0.0	-2.0	±	6.6	65.4	±	7.6	95.4	±	12.7	135.8	±	36.3
Oxazepam	6.0	±	7.2	4.4	±	9.6	1.9	±	8.2	1.5	±	3.9	-2.6	±	2.7	5.3	±	3.3
Chlordiazepoxide	-8.7	±	5.1	-11.4	±	11.1	-19.4	±	9.6	-12.5	±	2.8	-20.8	±	8.6	-51.3	±	0.4
Antidepressants																		
Dosulepin	-23.7	±	11.3	-54.2	±	3.6	-77.1	±	5.7	-22.5	±	1.2	-73.3	±	3.1	-83.8	±	7.4
Amitriptyline	-7.4	±	3.2	-41.2	±	0.3	-72.6	±	3.3	-11.6	±	8.8	-68.8	±	4.1	-81.9	±	6.7
Nortriptyline	-28.0	±	8.8	-57.6	±	0.5	-85.0	±	0.0	-16.8	±	3.2	-80.5	±	4.0	-88.1	±	2.6
Fluoxetine	8.3	±	6.7	1.1	±	14.8	-4.8	±	24.4	1.8	±	7.0	-10.7	±	8.3	-11.4	±	5.0
Norfluoxetine	8.7	±	10.1	-25.0	±	20.3	3.8	±	1.9	-15.7	±	0.6	-23.1	±	5.4	4.8	±	4.8
Venlafaxine	-6.5	±	0.7	-1.0	±	1.9	0.7	±	11.4	-2.9	±	3.6	1.5	±	5.0	18.3	±	9.0
Dissociative anaesthetics																		
Phencyclidine	-10.5	±	2.1	-8.3	±	1.0	-1.6	±	1.2	-0.1	±	12.6	-6.0	±	5.6	2.5	±	0.4
Ketamine	4.5	±	0.8	2.2	±	5.4	4.8	±	2.4	-5.0	±	5.8	-0.1	±	0.5	1.6	±	1.8
Norketamine	2.1	±	1.3	-0.1	±	0.9	1.9	±	1.4	-3.8	±	7.1	5.3	±	5.2	2.8	±	7.4
Other																		
Methaqualone	-3.1	±	4.0	7.4	±	3.3	5.9	±	11.5	-10.2	±	11.7	-27.6	±	6.7	-24.5	±	7.8
Sildenafil	3.9	±	0.2	-3.0	±	1.6	-40.2	±	11.8	13.5	±	2.7	-24.6	±	16.3	-43.4	±	26.7
Drug precursors																		
Ephedrine	-16.1	±	1.6	-27.5	±	5.6	-25.4	±	7.2	4.0	±	0.1	0.6	±	5.9	-3.4	±	11.3
Norephedrine	-40.2	±	20.2	-59.6	±	1.2	-64.5	±	0.0	-4.4	±	0.6	-29.7	±	3.8	-39.0	±	4.0

Table 6 - Percentage of analytes after three days reporting a stability change greater than 15 %

WWTP influent storage condition	Percentage of analytes with stability change > 15 %		
	12 hours	24 hours	72 hours
Raw (filtered) wastewater, pH 2, 2 ˚C	2	8	25
Raw (filtered) wastewater, pH 7.4, 2 ˚C	23	35	45
Raw (filtered) wastewater, pH 2, 19 ˚C	2	8	17
Raw (filtered) wastewater, pH 7.4, 19 ˚C	20	42	58
Raw (unfiltered) wastewater, pH 7.4, 2 ˚C	34	43	54
Raw (unfiltered) wastewater, pH 7.4, 19 ˚C	35	46	69

Table 7 – Summary of recoveries obtained with each parameter

Key (recovery change):	≤± 15 %	>± 15 %	>±30 %	>±50 %

Compound	Range of recoveries obtained with each parameter (%)				
	Stability[a]	Vaccum filtration[b]	Evaporation in silanised/non-silanised vials[c]	Evaporation temperature[d]	Pre LC-MS/MS filter[e]
Stimulants					
Cocaine	1 - -28	101 - 98	98 - 64	107 - 90	101 - 98
Benzoylecgonine	17 - -9	101 - 94	101 - 95	110 - 92	99 - 96
Norbenzoylecgonine	3 - -11	103 - 97	96 - 81	104 - 81	99 - 94
Norcocaine	0 - -31	106 - 100	99 - 73	103 - 90	101 - 96
Cocaethylene	0 - -19	109 - 102	95 - 70	108 - 90	101 - 98
Anhydroecgonine methyl ester	53 - -14	104 - 99	78 - 28	93 - 40	103 - 91
Ecgonidine	83 - -10	105 - 100	117 - 44	78 - 44	101 - 74
Amphetamine	88 - -11	110 - 97	88 - 16	96 - 51	103 - 96
Methamphetamine	12 - -14	108 - 99	89 - 23	95 - 59	103 - 99
Methcathinone	6 - -78	105 - 99	67 - 16	85 - 12	101 - 98
BZP	115 - -4	98 - 75	86 - 37	97 - 60	104 - 97
TFMPP	39 - -50	106 - 101	90 - 37	100 - 70	103 - 98
Hallucinogens					
MDA	10 - -11	104 - 101	99 - 41	102 - 83	104 - 98
MDMA	5 - -11	102 - 98	96 - 40	102 - 84	101 - 98
MDEA	5 - -12	103 - 98	97 - 35	106 - 81	101 - 98
MBDB	-1 - -22	105 - 102	93 - 27	97 - 75	101 - 97
BDB	3 - -38	108 - 102	92 - 27	96 - 73	100 - 96
Mescaline	-1 - -27	105 - 96	96 - 76	112 - 82	101 - 97
LSD	3 - -20	109 - 98	93 - 87	108 - 90	101 - 97
O-H-LSD	83 - -14	105 - 99	96 - 80	108 - 92	99 - 96
Human indicators					
Caffeine	48 - -15	103 - 99	99 - 36	108 - 81	103 - 96
1,7-dimethylxanthine	53 - -41	106 - 94	117 - 91	144 - 91	101 - 98
Nicotine	4 - -17	107 - 24	50 - 10	75 - 18	105 - 93
Continine	208 - -17	104 - 101	89 - 44	96 - 53	108 - 77
Opioids and morphine derivatives					
Heroin	4 - -100	104 - 99	89 - 68	91 - 34	98 - 95
6-acetylmorphine	-1 - -77	106 - 98	118 - 91	164 - 91	103 - 98
Codeine	24 - -4	104 - 97	99 - 73	99 - 73	102 - 99
Norcodeine	5 - -10	108 - 95	97 - 68	101 - 68	101 - 97
Oxycodone	13 - -9	104 - 98	82 - 67	89 - 1	102 - 99

Oxymorphone	43 - -12	104 - 100	102 - 46	92 - 1	102 - 80
Morphine	89 - -9	105 - 97	110 - 58	103 - 58	102 - 83
Normorphine	14 - -7	105 - 96	104 - 57	93 - 57	103 - 75
Dihydrocodeine	9 - -8	102 - 95	100 - 77	103 - 77	102 - 96
Buprenorphine	4 - -17	110 - 103	89 - 78	96 - 81	103 - 96
Norbuprenorphine	17 - -8	105 - 99	97 - 88	102 - 88	102 - 98
Methadone	4 - -23	109 - 103	90 - 50	95 - 79	102 - 97
EDDP	4 - -72	99 - 95	89 - 16	97 - 80	101 - 96
EMDP	33 - -41	102 - 97	64 - 1	91 - 17	99 - 95
Fentanyl	3 - -62	103 - 95	98 - 90	102 - 90	102 - 98
Norfentanyl	21 - -23	106 - 103	98 - 87	103 - 87	99 - 96
Propoxyphene	25 - -19	109 - 95	96 - 45	115 - 77	101 - 94
Norpropoxyphene	176 - -21	107 - 106	676 - 119	717 - 152	100 - 94
Tramadol	3 - -33	98 - 96	89 - 37	93 - 78	102 - 97
Nortramadol	11 - -63	103 - 100	98 - 81	109 - 87	102 - 98
Benzodiazepines					
Temazepam	19 - -63	105 - 96	90 - 86	106 - 79	101 - 97
Diazepam	-3 - -44	107 - 101	97 - 88	108 - 82	102 - 98
Nordiazepam	50 - -22	104 - 96	91 - 76	102 - 70	100 - 90
Nitrazepam	-10 - -100	96 - 96	80 - 66	95 - 44	95 - 82
7-aminonitrazepam	136 - -8	95 - 91	94 - 58	117 - 73	101 - 95
Oxazepam	8 - -29	104 - 98	101 - 86	116 - 80	100 - 95
Chlordiazepoxide	13 - -51	114 - 101	107 - 86	113 - 87	102 - 98
Antidepressants					
Dosulepin	5 - -84	105 - 95	90 - 47	92 - 47	103 - 98
Amitriptyline	10 - -82	104 - 91	90 - 45	93 - 58	102 - 98
Nortriptyline	-4 - -88	104 - 93	83 - 39	85 - 47	102 - 98
Fluoxetine	8 - -55	108 - 96	82 - 19	92 - 25	102 - 96
Norfluoxetine	9 - -56	105 - 96	74 - 19	81 - 27	102 - 98
Venlafaxine	18 - -44	109 - 101	100 - 61	104 - 91	101 - 97
Dissociative anaesthetics					
Phencyclidine	3 - -25	111 - 106	95 - 20	106 - 41	101 - 97
Ketamine	5 - -5	102 - 97	88 - 20	97 - 47	100 - 97
Norketamine	5 - -11	100 - 91	74 - 20	89 - 47	99 - 97
Other					
Methaqualone	7 - -28	114 - 101	94 - 21	109 - 63	97 - 92
Sildenafil	28 - -53	113 - 84	86 - 40	88 - 56	102 - 95

Drug precursors																				
Ephedrine/Pseudoephedrine	4	-	-57		105	-	102		100	-	32		105	-	59		100	-	97	
Norephedrine	5	-	-84		107	-	97		114	-	33		122	-	31		100	-	95	

[a]Stability of analytes in various storage conditions encountered during sample collection and storage

[b]Filtration of spiked river water through glass fibre filters (see supplementary material)

[c]Evaporation of SPE extracts in silanised and nonsilanised vials

[d]Evaporation of SPE extracts at temperatures 20 - 50 ˚C

[e]Recovery of compounds after filtration through various pre-LC-MS/MS filters (see supplementary material)

Supplementary material

Critical evaluation of methodology commonly used in sample collection, storage and preparation in the analysis of pharmaceuticals and illicit drugs in surface water and wastewater by solid phase extraction and liquid chromatography – mass spectrometry

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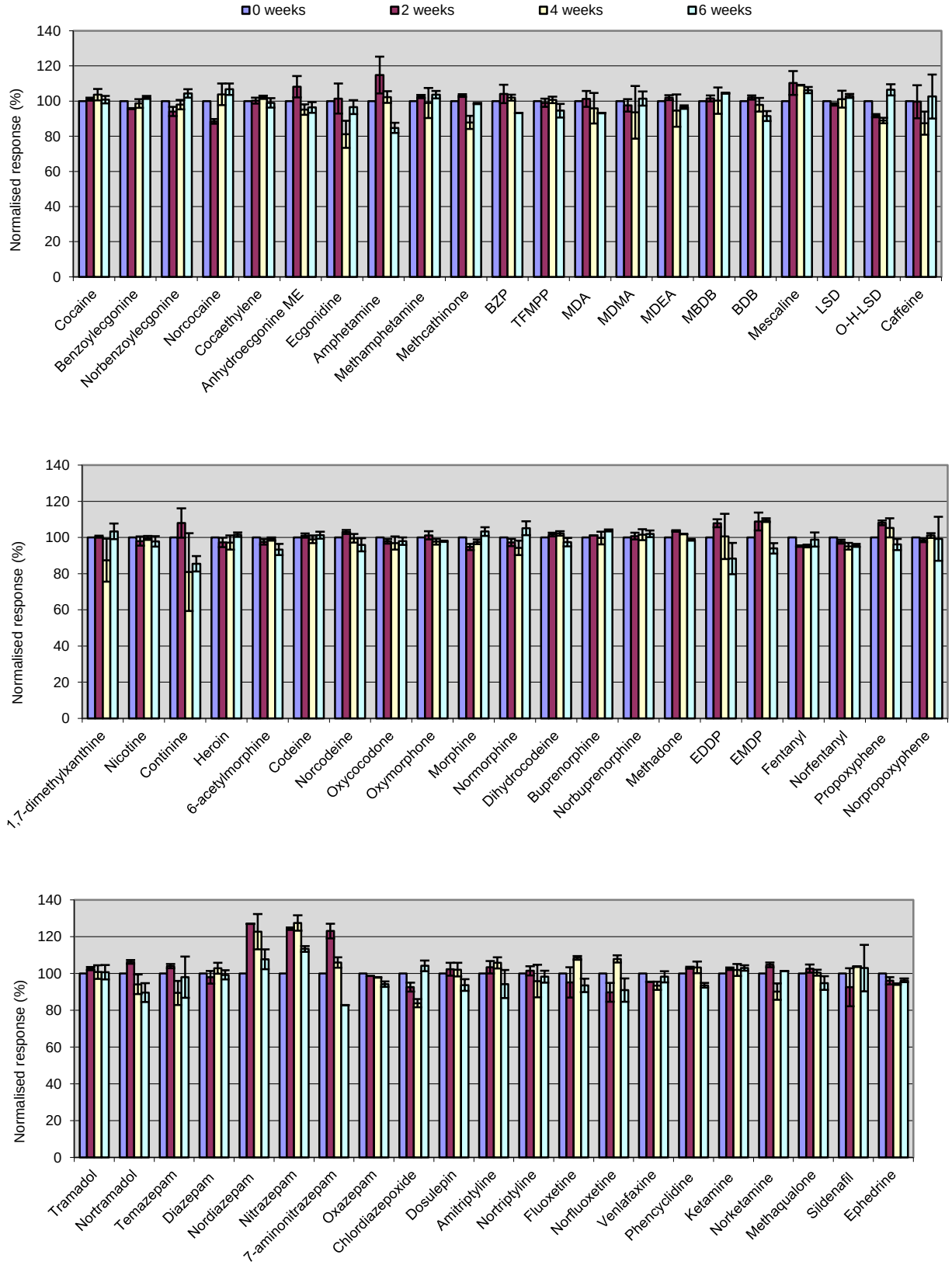

Figure S1 – Stability of analytes on Oasis MCX cartridges, extracted from the spiked WWTP influent, and stored at -20 ˚C over a 6 week period (n = 2 at each time-point)

Table S1 - Selected pharmaceuticals and their properties

Compound	CAS	Formula	MW	Pk_a		LogP		Supplier
				Experimental[a]	Calculated[b]	Experimental[a]	Calculated[b]	
Stimulants and their metabolites								
Cocaine	50-36-2	$C_{17}H_{21}NO_4$	303.4	8.6 (20°)	8.9	2.3	2.3	LGC
Benzoylecgonine	519-09-5	$C_{16}H_{19}NO_4$	289.3		10.8, 3.3	−1.3	2.3	LGC
Norbenzoylecgonine	60426-41-7	$C_{15}H_{17}NO_4$	275.3		10.4, 3.4		2.6	LGC
Norcocaine	N/A	$C_{16}H_{19}NO_4$	289.3		9.0		3.1	LGC
Cocaethylene	529-38-4	$C_{18}H_{23}NO_4$	317.4		9.0		2.8	LGC
Anhydroecgonine methyl ester	43021-26-7	$C_{10}H^{15}NO_2$	181.2		8.0		0.4	LGC
Ecgonidine	74242-55-0	$C_8H_{11}NO_2$	153.2		9.6, 3.8		1.5	LGC
Amphetamine	300-62-9	$C_9H_{13}N$	135.2	10.1	9.9	1.8	1.8	LGC
Methamphetamine	R-(-):33817-09-3, S-(+):537-46-2	$C_{10}H_{15}N$	149.2	10.1	10.4	2.1	2.2	LGC
Methcathinone	49656-78-2	$C_{10}H_{13}NO$	163.2		7.1		0.4	Sigma-Aldrich
BZP (Benzylpiperazine)	N/A	$C_{11}H_{16}N_2$	176.3		9.3, 3.4		1.1	LGC
TFMPP (Trifluoromethylphenylpiperazine)	N/A	$C_{11}H_{13}F_3N_2$	230.2		8.8, 2.1		1.3	LGC
Hallucinogens and their metabolites								
MDA (Methylenedioxyamphetamine)	4764-17-4	$C_{10}H_{13}NO_2$	179.2		9.9	1.64	1.6	LGC
MDMA (Methylenedioxymethamphetamine)	4254210-9	$C_{11}H_{15}NO_2$	193.2	(benzene, pH 9.0) 9.4	10.3		2.1	LGC
MDEA (Methylenedioxyethylamphetamine)	82801-81-8	$C_{12}H_{17}NO_2$	207.3		10.3		2.6	LGC
MBDB (Methylbenzodioxolylbutanamine)	145225-00-9	$C_{12}H_{17}NO_2$	207.3		10.5		2.6	LGC
BDB (Benzodioxolylbutanamine)	N/A	$C_{11}H_{15}NO_2$	193.2		10		2.2	LGC
Mescaline	832-92-8	$C_{11}H_{17}NO_3$	211.3	9.6	9.6	0.8	0.5	LGC
LSD (Lysergic acid diethylamide)	50-37-3	$C_{20}H_{25}N_3O$	323.4	7.5	7.4	2.9	2.7	LGC
O-H-LSD (2-oxo-3-hydroxy lysergic acid diethylamide)	N/A	$C_{20}H_{25}N_3O_3$	355.4		11.7, 6.8		-1.9	LGC
Human indicators								
Caffeine	58-08-2	$C_8H_{10}N_4O_2$	194.2	14.0 (25°), 10.4 (40°)	0.5	−0.07	-0.6	Sigma-Aldrich
1,7-dimethylxanthine	611-59-6	$C_7H_8N_4O_2$	180.2		8.5, 0.2		-0.9	Sigma-Aldrich
Nicotine	54-11-5	$C_{10}H_{14}N_2$	162.2	7.9, 3.2, (25°)	8.0, 3.2	1.2	0.6	Sigma-Aldrich

Continine	486-56-6	$C_{10}H_{12}N_2O$	176.2		4.7		0.07	Sigma-Aldrich
Creatinine	60-27-5	$C_4H_7N_3O$	113.1		6.9		-0.8	Fisher-Acros
Opioids, morphine derivatives and their metabolites								
Heroin	561-27-3	$C_{21}H_{23}NO_5$	369.4	7.6 (23°)	7.9	1.58	1.6	LGC
6-acetylmorphine	2784-73-8	$C_{19}H_{21}NO_4$	327.4		9.4, 8.0		1.6	LGC
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.4	8.2 (20°)	13.4, 8.2	0.6	1.4	Sigma-Aldrich
Norcodeine	467-15-2	$C_{17}H_{19}NO_3$	285.3	9.2 (25°)	13.3, 9.3	0.7	0.5	LGC
Oxycodone	76-42-6	$C_{18}H_{21}NO_4$	315.4	8.9 (20°)	13.1, 7.6	0.7	1.6	LGC
Oxymorphone	76-41-5	$C_{17}H_{19}NO_4$	301.3	9.3, 8.5	13.5, 9.2, 7.6	0	1.2	LGC
Morphine	57-27-2	$C_{17}H_{19}NO_3$	285.3	9.9, 8.0 (20°)	13.5, 9.5, 8.3	–0.1	0.9	LGC
Normorphine	466-97-7	$C_{16}H_{17}NO_3$	271.3	9.8 (25°)	13.4, 9.5, 9.2	−2.8	0.0	LGC
Dihydrocodeine	125-28-0	$C_{18}H_{23}NO_3$	301.4	8.8 (25°)	8.4		0.6	LGC
Buprenorphine	52485-79-7	$C_{29}H_{41}NO_4$	467.6	8.5, 10.0	9.5, 8.3	5.0	2.8	LGC
Norbuprenorphine	78715-23-8	$C_{25}H_{35}NO_4$	413.6		9.8, 9.1		1.2	LGC
Methadone	76-99-3	$C_{21}H_{27}NO$	309.4	8.94 (25°), 8.3 (20°)	9.1	3.9	3.9	Sigma-Aldrich
EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine)	66729-78-0	$C_{21}H_{25}N$	291.4		8.4		5.0	LGC
EMDP (2-ethyl-5-methyl-3,3-diphenylpyraline)	N/A	$C_{20}H_{23}N$	277.4		8.1		5.8	LGC
Fentanyl	437-38-7	$C_{22}H_{28}N_2O$	336.5		8.9, 0.3	2.3	3.7	LGC
Norfentanyl	N/A	$C_{14}H_{20}N_2O$	232.3		9.8, 0.3		1.7	LGC
Propoxyphene	469-62-5	$C_{22}H_{29}NO_2$	339.5	6.3	9.2	4.2	4.1	LGC
Norpropoxyphene	159208-83-0	$C_{21}H_{27}NO_2$	325.4		10.1		3.7	LGC
Tramadol	36282-47-0	$C_{16}H_{25}NO_2$	263.4	9.4, 8.3	9.6	3.0	2.3	Sigma-Aldrich
Nortramadol	N/A	$C_{15}H_{23}NO_2$	249.4		10.6		1.7	LGC
Benzodiazepines and their metabolites								
Temazepam	846-50-4	$C_{16}H_{13}ClN_2O_2$	300.7	1.6	11.7, 1.6	2.2	2.2	LGC
Diazepam	439-15-5	$C_{16}H_{13}ClN_2O$	284.7	3.3 (20°)	3.4	2.7	2.8	LGC
Nordiazepam	1088-11-5	$C_{15}H_{11}ClN_2O$	270.7	12.0, 3.5	11.7, 3.2	2.9	2.8	LGC
Nitrazepam	146-22-5	$C_{15}H_{11}N_3O_3$	281.3	10.8, 3.2 (20°)	11.4, 2.6	2.1	2.4	Sigma-Aldrich
7-aminonitrazepam	4928-02-3	$C_{15}H_{13}N_3O$	251.3		12.3, 4.3, 2.3		1.1	LGC

Oxazepam	604-75-1	$C_{15}H_{11}ClN_2O_2$	286.7	11.6, 1.7 (20°)	12.8, 10.9, 1.2	2.2	2.2	LGC
Chlordiazepoxide	58-25-3	$C_{16}H_{14}ClN_3O$	299.8	4.8	8.6, 6.5	2.4	2.8	LGC
Antidepressants and their metabolites								
Dosulepin	113-53-1	$C_{19}H_{21}NS$	295.4		9.1	2.8	4.3	LGC
Amitriptyline	549-18-8	$C_{20}H_{23}N$	277.4	9.4 (25°)	9.2	5.0	4.4	Sigma-Aldrich
Nortriptyline	894-71-3	$C_{19}H_{21}N$	263.4	9.7	10.0	1.7	4.0	Sigma-Aldrich
Fluoxetine	59333-67-4	$C_{17}H_{18}F_3NO$	309.3		10.1	1.8	3.9	LGC
Norfluoxetine	N/A	$C_{16}H_{16}F_3NO$	295.3		9.1		3.8	LGC
Venlafaxine	99300-78-4	$C_{17}H_{27}NO_2$	277.4		9.3	0.4	2.5	Sigma-Aldrich
Dissociative anesthetics and their metabolites								
PCP (phencyclidine)	77-10-1	$C_{17}H_{25}N$	243.4	8.5	8.2	4.7	4.3	LGC
Ketamine	1867-66-9	$C_{13}H_{16}ClNO$	237.7	7.5	6.5	3.1	3.0	Sigma-Aldrich
Norketamine	N/A	$C_{12}H_{14}ClNO$	223.7	6.7	6.3		2.4	LGC
Other								
Methaqualone	72-44-6	$C_{16}H_{14}N_2O$	250.3	2.5	3.0	4.3	2.5	LGC
Sildenafil	139755-83-23	$C_{22}H_{30}N_6O_4S$	474.6	8.7	6.0, 0.6		1.6	LGC
Drug precursors								
Ephedrine	50-98-6	$C_{10}H_{15}NO$	165.2	9.6 (25°)	9.5	1.1	1.0	LGC
Norephedrine	154-41-6	$C_9H_{13}NO$	151.2		12.1, 8.5		0.4	Sigma-Aldrich
Pseudoephedrine	345-78-8	$C_{10}H_{15}NO$	165.2	9.8	9.5	0.9	1.0	Sigma-Aldrich

[a] Moffat, A.C.; Osselton, D. M.; Widdop, B. Clarke's analysis of drugs and poisons, pharmaceutical press 2004, http://www.medicinescomplete.com/mc/clarke/current/, accessed June 2009
[b] ACD/I-lab accessed via ACD/chemsketch, version 12.0, Advanced chemistry development Inc. Toronto, ON, Canada. www.acdlabs.com

Table S2 – Absolute recovery of analytes in basic methanol after the evaporation of solvent

Compound	Evaporation from 7% (v/v) NH_4OH/MeOH, recovery (%) (n = 3)				
	Evaporation temperature				
	20 °C	30 °C	40 °C	50 °C	60 °C
Stimulants					
Cocaine	100 ± 5	96 ± 2	98 ± 4	92 ± 3	90 ± 2
Cocaine-d_3	100 ± 6	97 ± 1	97 ± 5	92 ± 2	92 ± 1
Benzoylecgonine	101 ± 5	94 ± 3	97 ± 7	92 ± 6	95 ± 2
Benzoylecgonine-d_8	103 ± 6	95 ± 5	97 ± 8	92 ± 2	93 ± 3
Ecgonine methyl ester	81 ± 5	78 ± 5	72 ± 4	45 ± 2	37 ± 9
Ecgonine methyl ester-d_3	92 ± 18	95 ± 11	84 ± 6	60 ± 6	43 ± 18
Norbenzoylecgonine	102 ± 2	93 ± 2	94 ± 4	91 ± 5	93 ± 3
Norcocaine	100 ± 5	98 ± 2	99 ± 7	90 ± 2	90 ± 3
Cocaethylene	96 ± 1	94 ± 1	95 ± 5	93 ± 3	90 ± 3
Cocaethylene-d_8	94 ± 1	96 ± 2	93 ± 4	95 ± 1	91 ± 1
Anhydroecgonine methyl ester	91 ± 5	93 ± 1	75 ± 1	56 ± 10	40 ± 2
Ecgonidine	78 ± 4	77 ± 3	67 ± 10	61 ± 2	57 ± 11
Amphetamine	85 ± 2	96 ± 4	88 ± 4	66 ± 12	51 ± 3
Amphetamine-d_{11}	92 ± 7	102 ± 2	95 ± 2	76 ± 14	61 ± 2
Methamphetamine	90 ± 7	95 ± 6	89 ± 1	68 ± 11	59 ± 5
Methamphetamine-d_{14}	92 ± 7	94 ± 2	85 ± 2	69 ± 12	58 ± 4
Methcathinone	84 ± 7	85 ± 8	67 ± 4	45 ± 12	32 ± 5
BZP	97 ± 7	93 ± 4	86 ± 2	78 ± 6	79 ± 7
TFMPP	98 ± 3	93 ± 1	90 ± 3	76 ± 6	70 ± 8
Hallucinogens					
MDA	99 ± 3	102 ± 3	99 ± 5	87 ± 4	87 ± 6
MDA-d_5	101 ± 7	96 ± 5	92 ± 7	83 ± 2	88 ± 4
MDMA	102 ± 5	99 ± 1	96 ± 6	88 ± 4	89 ± 8
MDMA-d_5	101 ± 7	98 ± 2	96 ± 8	89 ± 3	86 ± 3
MDEA	106 ± 3	96 ± 1	97 ± 5	88 ± 4	87 ± 4
MDEA-d_5	99 ± 4	98 ± 1	90 ± 6	88 ± 4	86 ± 4
MBDB	95 ± 3	95 ± 2	93 ± 2	78 ± 6	75 ± 8
MBDB-d_5	95 ± 3	95 ± 1	91 ± 4	83 ± 3	77 ± 6
BDB	94 ± 1	96 ± 2	92 ± 5	76 ± 4	73 ± 8
Mescaline	111 ± 8	96 ± 3	96 ± 9	83 ± 4	82 ± 4
Mescaline-d_9	107 ± 9	97 ± 6	91 ± 6	82 ± 3	82 ± 2
LSD	92 ± 1	90 ± 3	93 ± 3	92 ± 3	90 ± 2
LSD-d_3	91 ± 4	95 ± 1	92 ± 3	94 ± 2	92 ± 1
O-H-LSD	103 ± 1	102 ± 9	96 ± 7	92 ± 5	94 ± 3
Human indicators					
Caffeine	108 ± 4	96 ± 2	99 ± 6	89 ± 5	81 ± 8
Caffeine-d_9	105 ± 8	100 ± 3	95 ± 6	91 ± 5	83 ± 6
1,7-dimethylxanthine	118 ± 5	110 ± 4	111 ± 10	104 ± 4	108 ± 2
Nicotine	75 ± 16	71 ± 33	50 ± 13	21 ± 6	18 ± 13
Nicotine-d_4	72 ± 11	72 ± 32	50 ± 14	22 ± 7	19 ± 13
Continine	96 ± 6	91 ± 3	89 ± 12	80 ± 1	62 ± 11
Creatinine	20 ± 2	28 ± 12	40 ± 11	38 ± 4	29 ± 5
Opioids and morphine derivatives					
Heroin	72 ± 5	46 ± 1	68 ± 4	81 ± 4	81 ± 3
Heroin-d_9	84 ± 5	44 ± 2	69 ± 6	81 ± 5	81 ± 5
6-acetylmorphine	123 ± 3	134 ± 6	118 ± 8	105 ± 3	111 ± 2
Codeine	98 ± 2	97 ± 3	99 ± 9	96 ± 5	97 ± 3

Codeine-d_6	99	±	4	102	±	2	99	±	7	99	±	2	99	±	1
Norcodeine	101	±	4	98	±	3	97	±	6	92	±	6	93	±	1
Oxycodone	87	±	3	89	±	2	82	±	5	61	±	5	57	±	6
Oxycodone-d_6	94	±	2	95	±	0	85	±	7	70	±	5	64	±	5
Oxymorphone	92	±	5	88	±	3	83	±	9	67	±	7	56	±	1
Morphine	103	±	4	102	±	4	102	±	12	100	±	9	92	±	5
Morphine-d_6	103	±	6	101	±	3	99	±	11	100	±	8	88	±	9
Normorphine	93	±	2	90	±	2	92	±	10	90	±	6	81	±	5
Dihydrocodeine	103	±	4	99	±	2	100	±	8	101	±	4	100	±	2
Dihydrocodeine-d_6	96	±	7	97	±	7	96	±	7	93	±	1	96	±	3
Buprenorphine	90	±	3	90	±	0	89	±	1	88	±	5	88	±	4
Buprenorphine-d_4	88	±	4	90	±	1	85	±	4	87	±	5	88	±	4
Norbuprenorphine	96	±	2	96	±	1	95	±	4	92	±	4	92	±	4
Methadone	92	±	1	94	±	6	90	±	4	89	±	5	86	±	3
Methadone-d_9	91	±	2	96	±	2	90	±	6	91	±	2	88	±	3
EDDP	84	±	3	84	±	5	89	±	2	87	±	6	82	±	8
EDDP-d_3	85	±	4	84	±	2	89	±	0	89	±	5	85	±	6
EMDP	73	±	9	70	±	28	30	±	1	26	±	2	17	±	6
Fentanyl	97	±	3	98	±	3	98	±	4	96	±	3	100	±	2
Fentanyl-d_5	96	±	2	97	±	4	95	±	3	95	±	3	95	±	4
Norfentanyl	100	±	4	96	±	2	98	±	6	92	±	5	95	±	4
Propoxyphene	98	±	4	99	±	1	96	±	5	82	±	6	77	±	4
Propoxyphene-d_{11}	95	±	7	100	±	3	95	±	3	84	±	1	79	±	5
Norpropoxyphene	717	±	50	635	±	10	638	±	51	454	±	1	326	±	32
Norpropoxyphene-d_5	584	±	45	532	±	34	512	±	34	366	±	10	256	±	16
Tramadol	93	±	2	84	±	2	89	±	5	83	±	3	79	±	3
Nortramadol	101	±	9	100	±	11	98	±	13	87	±	8	95	±	9
Benzodiazepines															
Temazepam	94	±	3	96	±	3	89	±	3	81	±	7	79	±	7
Temazepam-d_5	93	±	5	95	±	6	86	±	3	80	±	8	76	±	8
Diazepam	95	±	4	91	±	1	88	±	5	83	±	6	82	±	10
Diazepam-d_5	94	±	3	94	±	3	87	±	2	83	±	6	83	±	9
Nordiazepam	92	±	6	88	±	2	85	±	2	74	±	6	70	±	6
Nitrazepam	87	±	5	76	±	5	66	±	3	54	±	3	44	±	5
7-aminonitrazepam	114	±	2	117	±	5	94	±	6	73	±	2	76	±	1
Oxazepam	91	±	8	94	±	2	87	±	4	80	±	6	80	±	8
Oxazepam-d_5	93	±	4	93	±	2	86	±	3	80	±	6	81	±	8
Chlordiazepoxide	113	±	1	108	±	1	107	±	7	99	±	5	105	±	7
Antidepressants															
Dosulepin	92	±	5	90	±	0	90	±	6	87	±	1	88	±	4
Amitriptyline	93	±	3	92	±	1	90	±	3	84	±	3	80	±	2
Nortriptyline	85	±	3	82	±	4	79	±	4	75	±	3	75	±	4
Fluoxetine	92	±	6	87	±	4	82	±	7	73	±	4	78	±	1
Fluoxetine-d_6	88	±	5	87	±	1	81	±	4	75	±	5	74	±	3
Norfluoxetine	80	±	2	81	±	2	74	±	5	66	±	5	66	±	1
Venlafaxine	99	±	3	100	±	4	100	±	5	92	±	5	91	±	4
Dissociative anesthetics															
Phencyclidine	89	±	5	97	±	2	86	±	6	58	±	14	41	±	5
PCP-d_5	84	±	7	93	±	3	80	±	3	59	±	14	43	±	7
Ketamine	95	±	5	93	±	7	84	±	3	65	±	8	47	±	1
Ketamine-d_4	94	±	4	92	±	2	80	±	1	63	±	10	44	±	1
Norketamine	86	±	4	89	±	9	74	±	3	57	±	4	48	±	8

Other															
Methaqualone	93	±	3	89	±	5	83	±	4	73	±	6	63	±	5
Methaqualone-d_7	94	±	2	90	±	2	86	±	6	81	±	5	72	±	4
Sildenafil	86	±	6	82	±	2	82	±	9	85	±	5	86	±	4
Drug precursors															
Ephedrine	103	±	11	105	±	5	100	±	7	83	±	5	89	±	6
Norephedrine	114	±	9	122	±	6	114	±	22	109	±	6	107	±	2

Table S3 – Absolute recovery of analytes in methanol after the evaporation of solvent

Compound	Evaporation from MeOH, recovery (%) (n=3)				
	Evaporation temperature				
	20°C	30°C	40°C	50°C	60°C
Stimulants					
Cocaine	99 ± 6	101 ± 1	95 ± 5	107 ± 6	105 ± 1
Cocaine-d_3	95 ± 3	96 ± 2	89 ± 4	101 ± 7	101 ± 2
Benzoylecgonine	97 ± 2	98 ± 0	95 ± 3	107 ± 3	110 ± 3
Benzoylecgonine-d_8	100 ± 5	101 ± 6	96 ± 4	110 ± 8	115 ± 5
Ecgonine methyl ester	108 ± 8	107 ± 7	100 ± 3	83 ± 19	112 ± 8
Ecgonine methyl ester-d_3	102 ± 8	106 ± 10	98 ± 9	72 ± 13	105 ± 5
Norbenzoylecgonine	86 ± 3	87 ± 0	81 ± 4	104 ± 12	100 ± 5
Norcocaine	99 ± 4	103 ± 2	95 ± 4	103 ± 4	103 ± 2
Cocaethylene	101 ± 3	103 ± 1	93 ± 2	108 ± 3	106 ± 3
Cocaethylene-d_8	93 ± 2	95 ± 1	90 ± 2	101 ± 7	101 ± 1
Anhydroecgonine methyl ester	88 ± 3	89 ± 2	78 ± 3	65 ± 11	66 ± 3
Ecgonidine	49 ± 1	48 ± 3	44 ± 1	55 ± 14	51 ± 2
Amphetamine	67 ± 8	74 ± 1	67 ± 3	66 ± 5	85 ± 3
Amphetamine-d_{11}	74 ± 9	81 ± 3	72 ± 2	72 ± 1	90 ± 2
Methamphetamine	85 ± 10	94 ± 2	81 ± 2	84 ± 11	95 ± 3
Methamphetamine-d_{14}	87 ± 7	93 ± 3	80 ± 2	81 ± 13	92 ± 2
Methcathinone	40 ± 3	46 ± 5	33 ± 2	31 ± 3	12 ± 1
BZP	70 ± 4	73 ± 1	60 ± 1	68 ± 10	63 ± 3
TFMPP	100 ± 3	96 ± 1	87 ± 3	89 ± 7	89 ± 3
Hallucinogens					
MDA	89 ± 6	94 ± 3	83 ± 3	93 ± 7	102 ± 3
MDA-d_5	86 ± 8	91 ± 4	79 ± 2	88 ± 5	98 ± 2
MDMA	92 ± 9	97 ± 3	84 ± 3	93 ± 5	98 ± 4
MDMA-d_5	91 ± 8	93 ± 5	83 ± 1	95 ± 7	97 ± 2
MDEA	88 ± 1	89 ± 2	81 ± 4	93 ± 4	93 ± 1
MDEA-d_5	85 ± 5	87 ± 3	78 ± 1	92 ± 9	91 ± 2
MBDB	90 ± 4	93 ± 0	88 ± 2	92 ± 9	97 ± 3
MBDB-d_5	89 ± 4	91 ± 2	85 ± 2	92 ± 7	94 ± 1
BDB	77 ± 6	83 ± 1	79 ± 3	86 ± 7	90 ± 5
Mescaline	108 ± 8	112 ± 4	94 ± 6	98 ± 5	105 ± 2
Mescaline-d_9	103 ± 5	107 ± 4	90 ± 1	97 ± 9	106 ± 1
LSD	96 ± 5	100 ± 2	93 ± 3	108 ± 6	101 ± 5
LSD-d_3	92 ± 6	94 ± 5	86 ± 1	102 ± 9	98 ± 2
O-H-LSD	106 ± 5	108 ± 3	96 ± 3	103 ± 5	104 ± 5
Human indicators					
Caffeine	96 ± 3	98 ± 6	90 ± 3	101 ± 10	99 ± 5
Caffeine-d_9	94 ± 4	95 ± 5	88 ± 4	99 ± 8	104 ± 0
1,7-dimethylxanthine	93 ± 2	96 ± 1	91 ± 4	121 ± 19	144 ± 3
Nicotine	61 ± 5	64 ± 2	48 ± 3	27 ± 7	26 ± 4
Nicotine-d_4	64 ± 5	67 ± 4	52 ± 2	30 ± 8	29 ± 3
Continine	56 ± 2	57 ± 1	53 ± 0	81 ± 1	70 ± 2
Creatinine	3 ± 0	3 ± 0	3 ± 0	18 ± 21	5 ± 0
Opioids and morphine derivatives					
Heroin	91 ± 1	90 ± 3	85 ± 4	90 ± 8	34 ± 0
Heroin-d_9	97 ± 2	92 ± 9	86 ± 1	90 ± 9	29 ± 1
6-acetylmorphine	93 ± 5	97 ± 1	91 ± 3	115 ± 11	164 ± 5
Codeine	77 ± 5	80 ± 2	73 ± 3	93 ± 22	85 ± 6

Codeine-d_6	74	±	5	77	±	2	71	±	3	99	±	23	83	±	1
Norcodeine	72	±	4	74	±	2	68	±	2	89	±	20	80	±	2
Oxycodone	80	±	5	85	±	2	67	±	2	63	±	21	1	±	0
Oxycodone-d_6	86	±	5	88	±	3	68	±	2	65	±	20	1	±	0
Oxymorphone	53	±	1	53	±	1	46	±	1	48	±	24	1	±	0
Morphine	59	±	3	61	±	1	58	±	1	82	±	24	74	±	2
Morphine-d_6	60	±	4	61	±	4	57	±	1	95	±	30	76	±	1
Normorphine	57	±	1	58	±	2	57	±	1	78	±	24	74	±	2
Dihydrocodeine	80	±	3	82	±	3	77	±	2	96	±	25	89	±	4
Dihydrocodeine-d_6	79	±	1	82	±	4	74	±	2	101	±	21	91	±	1
Buprenorphine	90	±	3	90	±	2	81	±	3	96	±	7	90	±	3
Buprenorphine-d_4	89	±	2	89	±	2	78	±	2	95	±	11	89	±	2
Norbuprenorphine	99	±	3	99	±	1	88	±	4	102	±	8	98	±	4
Methadone	87	±	1	89	±	2	79	±	5	95	±	7	85	±	1
Methadone-d_9	82	±	3	86	±	1	74	±	2	94	±	11	85	±	2
EDDP	97	±	2	96	±	2	85	±	3	80	±	7	82	±	1
EDDP-d_3	95	±	2	96	±	1	85	±	3	83	±	6	83	±	3
EMDP	91	±	4	90	±	7	64	±	6	25	±	2	28	±	1
Fentanyl	96	±	3	98	±	1	90	±	1	102	±	7	98	±	5
Fentanyl-d_5	101	±	3	101	±	3	90	±	3	104	±	12	101	±	2
Norfentanyl	92	±	2	93	±	1	87	±	2	99	±	5	103	±	2
Propoxyphene	109	±	7	115	±	2	92	±	2	105	±	9	97	±	4
Propoxyphene-d_{11}	103	±	6	110	±	5	93	±	3	103	±	8	98	±	2
Norpropoxyphene	187	±	63	261	±	8	246	±	14	517	±	154	152	±	9
Norpropoxyphene-d_5	167	±	57	224	±	9	200	±	8	418	±	115	111	±	8
Tramadol	85	±	4	89	±	2	83	±	3	90	±	6	78	±	2
Nortramadol	98	±	10	109	±	12	89	±	1	106	±	8	104	±	5
Benzodiazepines																
Temazepam	100	±	6	106	±	3	90	±	0	92	±	7	89	±	5
Temazepam-d_5	102	±	9	107	±	6	90	±	2	91	±	2	89	±	2
Diazepam	108	±	2	107	±	1	97	±	4	99	±	7	102	±	5
Diazepam-d_5	110	±	4	113	±	1	98	±	2	100	±	7	103	±	2
Nordiazepam	102	±	7	99	±	2	76	±	3	93	±	25	73	±	7
Nitrazepam	95	±	9	92	±	8	69	±	6	53	±	4	49	±	7
7-aminonitrazepam	109	±	8	103	±	6	82	±	7	93	±	16	100	±	5
Oxazepam	116	±	5	116	±	3	101	±	4	99	±	10	102	±	5
Oxazepam-d_5	116	±	5	118	±	3	99	±	4	97	±	8	102	±	2
Chlordiazepoxide	99	±	3	98	±	2	87	±	1	96	±	5	94	±	4
Antidepressants																
Dosulepin	55	±	4	55	±	4	47	±	4	89	±	27	58	±	5
Amitriptyline	65	±	6	63	±	4	58	±	3	84	±	16	63	±	3
Nortriptyline	58	±	10	53	±	5	47	±	5	83	±	20	52	±	3
Fluoxetine	29	±	7	26	±	4	25	±	4	55	±	31	30	±	1
Fluoxetine-d_6	24	±	6	22	±	4	21	±	2	49	±	32	26	±	2
Norfluoxetine	33	±	10	27	±	5	27	±	4	57	±	32	33	±	1
Venlafaxine	98	±	5	100	±	2	95	±	6	102	±	2	104	±	3
Dissociative anesthetics																
Phencyclidine	100	±	5	106	±	4	95	±	3	81	±	20	86	±	4
PCP-d_5	96	±	11	107	±	4	97	±	3	82	±	20	87	±	5
Ketamine	95	±	2	97	±	0	88	±	2	79	±	9	83	±	4
Ketamine-d_4	92	±	3	94	±	1	84	±	3	76	±	11	79	±	2
Norketamine	78	±	4	77	±	5	63	±	3	61	±	13	47	±	1

Other										
Methaqualone	109	±	3	109	±	3	94	±	4	95	±	10	90	±	5
Methaqualone-d_7	113	±	6	113	±	7	102	±	3	102	±	5	103	±	1
Sildenafil	62	±	2	65	±	4	56	±	5	88	±	19	68	±	6
Drug precursors										
Ephedrine	63	±	6	68	±	3	59	±	5	73	±	8	78	±	3
Norephedrine	31	±	6	33	±	4	33	±	2	71	±	43	54	±	3

Table S4 - Stability study - Concentration of compounds in control wastewater sample at time-point zero

Compound	Concentration of compound at time-point 0 (ng L^{-1} ± SD)		
Stimulants			
Cocaine	77.4	±	5.4
Benzoylecgonine	519.5	±	35.3
Norbenzoylecgonine	18.4	±	1.6
Norcocaine	<MQL		
Cocaethylene	6.9	±	0.5
Anhydroecgonine methyl ester	<MQL		
Ecgonidine	<MQL		
Amphetamine	15.9	±	1.4
Methamphetamine	<MQL		
Methcathinone	<MQL		
BZP	46.1	±	0.7
TFMPP	10.0	±	0.9
Hallucinogens			
MDA	<MQL		
MDMA	55.0	±	6.2
MDEA	<MQL		
MBDB	<MQL		
BDB	<MQL		
Mescaline	<MQL		
LSD	<MQL		
O-H-LSD	<MQL		
Human indicators			
Caffeine	52419.2	±	694.2
1,7-dimethylxanthine	30059.8	±	107.2
Nicotine	4589.9	±	36.8
Continine	1.0	±	1.1
Opioids and morphine derivatives			
Heroin	<MQL		
6-acetylmorphine	2.1	±	0.3
Codeine	1951.3	±	28.9
Norcodeine	67.4	±	3.0
Oxycodone	8.4	±	1.0
Oxymorphone	8.0	±	0.7
Morphine	408.3	±	8.2
Morphine-3β-glucuronide	177.1	±	1.9
Normorphine	132.3	±	10.0
Dihydrocodeine	189.3	±	16.8
Buprenorphine	<MQL		
Norbuprenorphine	<MQL		
Methadone	58.9	±	4.4
EDDP	35.7	±	2.3
EMDP	<MQL		
Fentanyl	<MQL		
Norfentanyl	<MQL		
Propoxyphene	<MQL		
Norpropoxyphene	374.7	±	33.7
Tramadol	968.2	±	31.2
Nortramadol	86.1	±	7.8

Benzodiazepines			
Temazepam	140.7	±	2.2
Diazepam	0.0	±	0.0
Nordiazepam	10.0	±	1.1
Nitrazepam	<MQL		
7-aminonitrazepam	<MQL		
Oxazepam	34.4	±	1.4
Chlordiazepoxide	<MQL		
Antidepressants			
Dosulepin	60.0	±	2.8
Amitriptyline	122.7	±	7.5
Nortriptyline	<MQL		
Fluoxetine	36.9	±	0.4
Norfluoxetine	10.6	±	0.6
Venlafaxine	124.7	±	9.2
Dissociative anesthetics			
Phencyclidine	<MQL		
Ketamine	96.5	±	6.0
Norketamine	16.5	±	1.0
Other			
Methaqualone	<MQL		
Sildenafil	30.3	±	3.7
Drug precursors			
Ephedrine	449.3	±	18.4
Norephedrine	<MQL		

This section discusses the following sample preparation parameters: (i) the effect of SPE sorbent; (ii) recovery of analytes during vacuum filtration through glass fibre filters and (iii) pre LC-MS filter membranes.

1. EXPERIMENTAL

1.1 Solid phase extraction sorbent

To assess the recovery efficiency of Oasis® HLB and MCX SPE sorbents (3 cm^3, 60 mg), UHQ water (100 mL) was spiked with 50 ng of each compound, with exception of norpropoxyphene and creatinine at 100 ng, and cannabinoids at 150 ng. The sample was extracted using the protocols described below. After elution into silanised vials, extracts were subsequently evaporated at 40 °C under a stream of nitrogen and reconstituted in 0.3 % CH_3COOH, 5 % $MeOH/H_2O$ (v/v) (500 µl).

Oasis HLB protocol

Oasis HLB (60 mg, Waters, UK) cartridges were conditioned with methanol (2 ml, flow rate 3 ml min^{-1}) followed by equilibration with water (2 ml, pH 7, flow rate 3 ml min^{-1}). Then, 100 mL of sample (pH 7, adjusted with NaOH) was passed through each cartridge (flow rate 6 ml min^{-1}). Elution was performed with methanol (4 mL, flow rate 3 ml min^{-1}) into silanised vials.

Oasis MCX protocol

Oasis MCX (60 mg, Waters, UK) cartridges were conditioned with methanol (2 ml, flow rate 3 ml min^{-1}) followed by equilibration with 2 % (v/v) $HCOOH/H_2O$ (2 ml, pH 1.8, flow rate 3 ml min^{-1}). Then, 100 mL of sample (pH 1.8, adjusted with HCl) was passed through each cartridge (flow rate 6 ml min^{-1}). Elution was performed with methanol (2 mL) and 7 % (v/v) $NH_4OH/MeOH$ (2mL, 3 ml min^{-1}) into silanised vials.

1.2 Syringe filters membrane investigation

Filtration of samples prior to injection into the UPLC system is vital in order to maintain guard columns and sub-2 µm analytical columns. Various different filter membranes (see list below) were assessed to ensure that target analytes were not removed. Compounds were spiked into sample diluent, 0.3 % CH_3COOH, 5 % $MeOH/H_2O$ (v/v), at a concentration of 50 µg L^{-1}, with exception of norpropoxyphene and creatinine at 100 µg L^{-1} and cannabinoids at 150 µg L^{-1}. Aliquots of this sample (500 µL) were then filtered through each of the membranes (each membrane was discarded after one 500 µL sample) and analysed by LC-MS/MS. The evaluated membranes were as follows:

- Phenex – RC (Phenomenex, UK): Diameter 4mm, 0.2µm membrane; membrane, hydrophilic regenerated cellulose; application, broad range of aqueous and mixed-organic solutions
- Phenex – PTFE (Phenomenex, UK): Diameter 4mm, 0.2µm membrane; membrane, hydrophobic PTFE; application, organic based, highly acidic or basic samples and solvents
- Phenex – NY (Phenomenex, UK): Diameter 4mm, 0.2µm membrane; membrane inherently hydrophilic; application, many aqueous and mixed organic samples
- IC Millex – LG (Millipore, UK): Diameter 13mm, 0.2µm membrane; membrane, hydrophilic PTFE; application, aqueous and mild organic solutions for ion chromatography
- Millex – LG (Millipore, UK): Diameter 4mm, 0.2µm membrane; membrane, hydrophilic PTFE; application aqeous and organic solutions
- Millex – GV (Millipore, UK); Diameter 4mm, 0.22µm membrane; membrane hydrophilic PVDF, application aqueous and mild organic solutions
- Whatman – PTFE (Whatman, UK): Diameter 13mm, 0.2µm membrane; membrane hydrophobic PTFE; application, organic based samples

1.3 Filtration of environmental samples

The potential removal of compounds during vacuum filtration was investigated. Filters assessed were GF/D (2.7 µm), GF/C (1.2 µm) and GF/F (0.7 µm) (Whatman, UK). UHQ water (100ml) was spiked with 50 ng of each compound, except norpropoxyphene and creatinine at 100 ng, and cannabinoids at 150 ng. Each sample was passed through one filter only, before undergoing the SPE procedure with Oasis® MCX sorbent as discussed in section 0

2 RESULTS AND DISCUSSION

2.1 Solid phase extraction sorbent

Optimisation of SPE sorbents involved the Oasis HLB and Oasis MCX. Overall, the recovery of the majority of compounds was similar with both sorbent protocols. Recoveries are presented for all compounds in **Figure S2**. Key differences included the ability of the MCX to extract ecgonine methyl ester, anhydroecgonine methyl ester and ecgonidine, whereas, the HLB protocol provided higher recoveries for benzodiazepines and heroin (the higher recovery is more likely a result of the extraction solvent as discussed previously, see main manuscript section 3.1). An additional advantage of the Oasis MCX is the need to adjust samples to an acidic pH for extraction. Adjustment to an acidic pH has been shown to promote the stability of nearly all compounds. Furthermore, as compounds are retained through ion –exchange interactions using the MCX, it is possible to incorporate more effective wash steps that could lower matrix effects as oppose to the HLB [1]. The ability to extract the cocaine metabolites, incorporate a wash step, and promote stability of samples resulted in the Oasis MCX sorbent being selected for future work. The impact of the insufficient recovery of heroin can be overcome to some extent with the inclusion of its metabolites 6-acetylmorphine, while the recovery of benzodiazepines was higher with the HLB, it was still above or around 80 % with the MCX.

2.2 Syringe filter membrane investigation

Filtration of samples to remove particulates is a well known and well documented preventative measure in liquid chromatography [2]. Although syringe filters may remove non-dissolved particulates and protect the chromatographic system, they could also be responsible for the adsorption of target analytes dissolved in the sample of interest [3]. Bijlsma et al. [4] investigated the use of 0.2 µm polypropylene and PTFE (manufacturer not reported in the manuscript) filter membranes, however this group reported recoveries <70 % for cocaine, cocaethylene, norcocaine and THC-COOH. Other groups have reported the use of PTFE filters [5,6] and GHP [7], but recoveries from these membranes were not reported in the respective manuscripts. To determine a suitable syringe filter for the suite of compounds selected in this study, a range of different membranes were investigated (see section 1.2).

The majority of compounds achieved a recovery greater than 90 % with all membranes evaluated. However, there were a small number of compounds below this threshold and these compounds have been highlighted in **Table S5**. Low recoveries of 74, 75 and 84 % were found for ecgonidine using the Phenomenex RC, and Millipore PTFE and LG respectively. The same three filters were also responsible for low recovery of creatinine, oxymorphone and normorphine. The recovery of morphine was below 90 % for both Millipore RC and LG. The highest removal of compounds was associated with THC and THC-COOH using Phenomenex nylon, Millipore PTFE, LG, PVDF and Whatman PTFE. Of the evaluated syringe filters, only the Phenomenex PTFE achieved a recovery > 90 % for all studied compounds. For this reason, the filter was selected for further method development. However it was found that when tested with wastewater samples the filter was only able to filter a very small quantity of the 500 µL sample before it blocked. The blockage of the filter was more likely a result of the filter diameter (4 mm) rather than the membrane itself. Utilisation of a larger diameter would have been likely to have solved this issue. Nevertheless, as the Whatman PTFE (13 mm) was readily available in the laboratory and recovered all but one compound > 90 % (nitrazepam, 85 %), this filter

was tested with wastewater and found to be suitable (THC-COOH and THC were not quantified in the final methodology).

2.3 Environmental sample filtration

After the collection of environmental samples, the particulates which are contained in the sample must be removed to make the sample amenable to the next analytical step. All procedures that use SPE for extraction employ vacuum filtration beforehand (see main manuscript **Table 1**). This is typically achieved by filtering samples through glass microfibre filters. To ensure that the vacuum filtration did not lead to the removal of analytes, three glass fibre filters with different pore sizes were evaluated. **Table S6** lists the recoveries of compounds after filtration. Recoveries were calculated against the same sample that had undergone the same analytical procedure, but without the vacuum filtration of samples. The vacuum filtration of samples through glass microfibre filters did not influence the recovery of target analytes; therefore the spiking of internal standards after filtration is acceptable.

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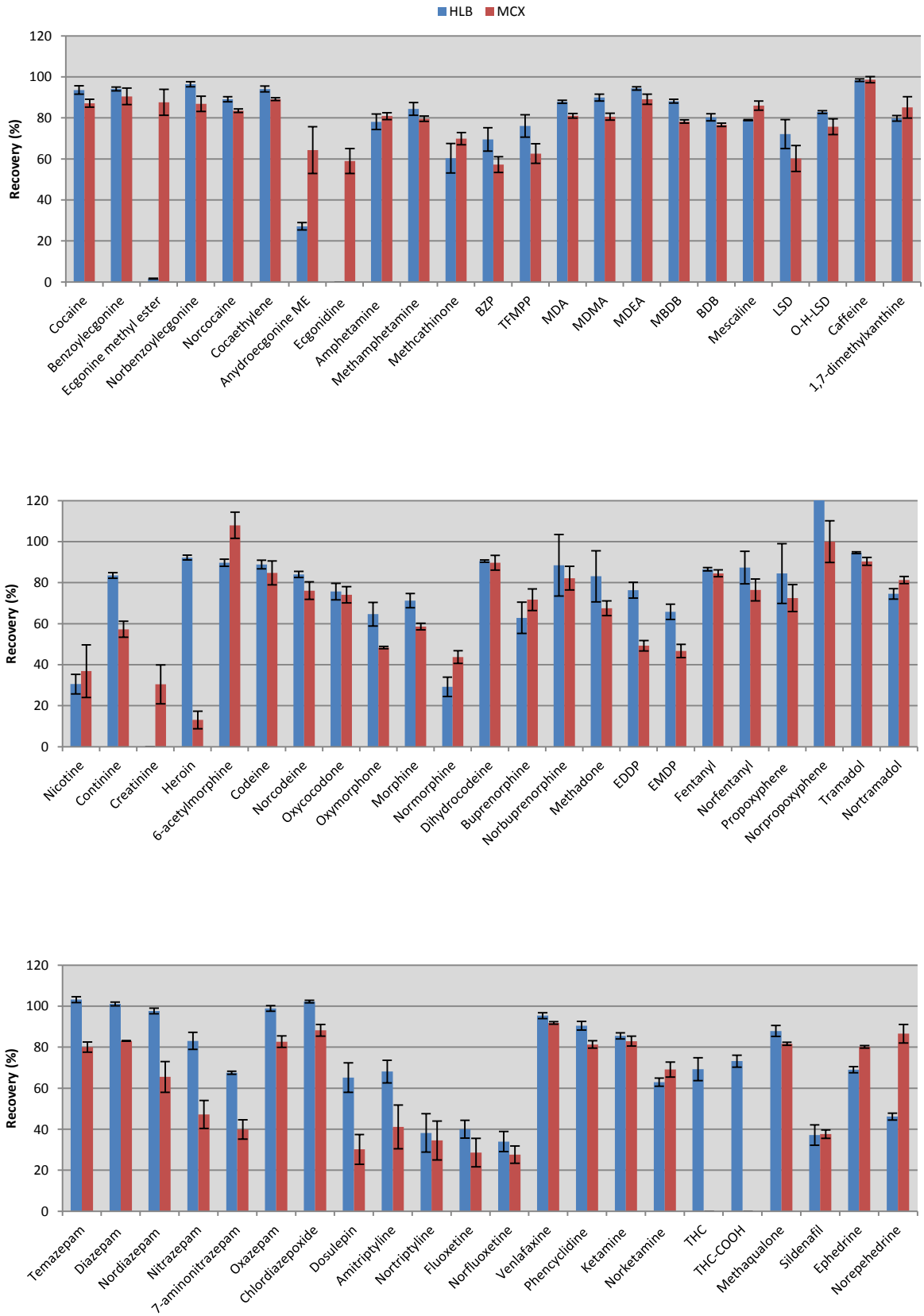

Figure S2 – SPE sorbent evaluation, total recovery of Oasis HLB and MCX for all compounds from UHQ water (n = 3)

Table S5 – Filter membrane investigation (recoveries < 90 % highlighted)

Compound	Syringe filter membrane recovery (%) (n=2)													
	Phenomenex Nylon		Phenomenex PTFE		Phenomenex RC		Millipore PTFE		Millipore LG		Millipore PVDF		Whatman PTFE	
Stimulants														
Cocaine	100	± 0	100	± 1	100	± 3	101	± 1	100	± 0	98	± 1	98	± 0
Cocaine-d_3	100	± 2	98	± 2	100	± 2	100	± 3	99	± 1	98	± 2	97	± 1
Benzoylecgonine	99	± 2	97	± 2	98	± 0	99	± 2	96	± 1	99	± 0	98	± 3
Benzoylecgonine-d_8	98	± 0	97	± 1	100	± 0	100	± 1	98	± 1	101	± 0	101	± 3
Ecgonine methyl ester	104	± 2	98	± 1	87	± 0	99	± 0	99	± 2	96	± 3	101	± 1
Ecgonine methyl ester-d_3	100	± 1	98	± 1	98	± 3	102	± 1	99	± 0	100	± 7	102	± 2
Norbenzoylecgonine	99	± 1	97	± 1	96	± 1	99	± 1	94	± 1	98	± 3	98	± 1
Norcocaine	101	± 1	99	± 1	98	± 1	99	± 1	96	± 0	100	± 0	98	± 4
Cocaethylene	101	± 1	99	± 1	98	± 1	100	± 3	99	± 2	98	± 0	98	± 1
Cocaethylene-d_8	99	± 2	100	± 2	101	± 3	100	± 2	99	± 1	98	± 1	98	± 0
Anhydroecgonine methyl ester	103	± 2	98	± 2	91	± 7	98	± 4	96	± 3	100	± 4	100	± 0
Ecgonidine	99	± 3	101	± 1	74	± 6	75	± 8	84	± 7	93	± 2	100	± 2
Amphetamine	99	± 1	97	± 1	103	± 2	99	± 3	96	± 1	102	± 3	101	± 2
Amphetamine-d_{11}	98	± 1	98	± 0	99	± 1	102	± 1	100	± 2	98	± 3	96	± 3
Methamphetamine	103	± 1	99	± 1	100	± 1	100	± 1	99	± 0	100	± 1	100	± 0
Methamphetamine-d_{14}	99	± 1	99	± 0	101	± 0	100	± 1	98	± 1	98	± 3	100	± 0
Methcathinone	100	± 1	99	± 1	100	± 0	101	± 1	98	± 1	101	± 0	100	± 1
BZP	104	± 7	103	± 6	97	± 8	99	± 6	104	± 4	104	± 7	102	± 3
TFMPP	101	± 2	101	± 1	98	± 3	102	± 1	98	± 1	101	± 2	103	± 0
Hallucinogens														
MDA	104	± 1	99	± 1	98	± 1	99	± 3	100	± 1	101	± 0	102	± 1
MDA-d_5	99	± 2	99	± 1	101	± 1	98	± 2	100	± 0	100	± 3	100	± 1
MDMA	101	± 3	100	± 3	100	± 1	99	± 1	98	± 1	100	± 0	100	± 0
MDMA-d_5	99	± 0	99	± 3	100	± 0	99	± 1	96	± 1	99	± 1	101	± 0
MDEA	101	± 0	98	± 0	100	± 1	101	± 2	99	± 0	100	± 2	100	± 3
MBDB	98	± 1	98	± 1	100	± 1	101	± 0	97	± 1	99	± 3	99	± 3
MBDB-d_5	100	± 0	98	± 0	99	± 4	101	± 1	98	± 1	99	± 0	102	± 2
BDB	99	± 0	96	± 2	97	± 1	98	± 0	97	± 1	98	± 1	100	± 0
Mescaline	100	± 1	99	± 3	101	± 1	97	± 0	97	± 4	100	± 1	101	± 1

Mescaline-d_9	100	±	2	99	±	1	98	±	3	100	±	1	99	±	1	99	±	0	101	±	1
LSD	100	±	1	100	±	2	101	±	0	100	±	1	99	±	1	98	±	1	97	±	3
LSD-d_3	100	±	2	98	±	2	97	±	2	97	±	3	99	±	2	98	±	2	101	±	1
O-H-LSD	98	±	3	98	±	0	98	±	1	98	±	2	96	±	0	98	±	1	99	±	0
Human indicators																						
Caffeine	101	±	3	98	±	1	100	±	0	102	±	2	96	±	3	101	±	2	103	±	2
Caffeine-d_9	98	±	2	99	±	1	98	±	1	98	±	3	97	±	2	99	±	0	100	±	1
1,7-dimethylxanthine	101	±	2	98	±	1	99	±	1	100	±	1	99	±	1	100	±	2	99	±	1
Nicotine	95	±	13	101	±	12	98	±	13	103	±	11	93	±	6	105	±	15	99	±	14
Nicotine-d_4	100	±	15	106	±	12	99	±	13	101	±	13	88	±	4	106	±	11	99	±	13
Continine	99	±	0	98	±	1	108	±	2	78	±	14	77	±	13	92	±	0	96	±	0
Creatinine	106	±	5	102	±	2	64	±	7	60	±	8	81	±	4	87	±	5	104	±	3
Opioids and morphine derivatives																						
Heroin	98	±	0	97	±	1	98	±	2	98	±	0	95	±	1	98	±	2	98	±	1
Heroin-d_9	102	±	4	99	±	2	100	±	2	101	±	1	98	±	2	95	±	1	95	±	4
6-acetylmorphine	103	±	2	99	±	1	99	±	0	99	±	1	100	±	1	99	±	2	98	±	2
Codeine	102	±	3	99	±	1	100	±	1	102	±	1	100	±	1	100	±	1	100	±	2
Codeine-d_6	100	±	8	101	±	0	99	±	1	101	±	1	100	±	1	100	±	0	99	±	1
Norcodeine	99	±	1	97	±	2	97	±	0	101	±	1	99	±	3	101	±	2	101	±	2
Oxycodone	100	±	1	102	±	0	101	±	0	100	±	0	99	±	1	99	±	0	99	±	1
Oxycodone-d_6	103	±	2	100	±	0	100	±	0	101	±	2	100	±	1	100	±	0	100	±	0
Oxymorphone	102	±	3	96	±	1	80	±	6	84	±	7	85	±	6	99	±	3	99	±	2
Morphine	102	±	2	98	±	1	91	±	10	85	±	5	83	±	8	98	±	2	100	±	0
Morphine-d_6	99	±	2	100	±	1	83	±	11	77	±	9	72	±	5	88	±	7	96	±	6
Normorphine	103	±	1	100	±	0	75	±	8	77	±	8	77	±	10	96	±	2	99	±	3
Dihydrocodeine	101	±	0	99	±	1	99	±	1	102	±	2	96	±	2	100	±	1	99	±	1
Dihydrocodeine-d_6	101	±	1	99	±	2	98	±	1	99	±	0	99	±	3	100	±	0	99	±	0
Buprenorphine	103	±	1	99	±	0	98	±	2	100	±	1	96	±	0	100	±	1	100	±	1
Buprenorphine-d_4	105	±	1	100	±	1	101	±	1	101	±	0	100	±	1	98	±	2	99	±	1
Norbuprenorphine	102	±	1	99	±	1	98	±	1	101	±	1	98	±	0	98	±	0	98	±	3
Methadone	101	±	2	98	±	3	99	±	0	102	±	1	97	±	1	98	±	1	101	±	2
Methadone-d_9	98	±	2	99	±	1	98	±	0	99	±	0	95	±	0	98	±	2	98	±	3
EDDP	101	±	1	99	±	1	99	±	0	101	±	0	98	±	1	96	±	4	98	±	1

EDDP-d_3	103	±	1	100	±	0	99	±	0	102	±	1	100	±	0	97	±	3	98	±	1
EMDP	99	±	3	98	±	1	97	±	1	98	±	2	95	±	2	98	±	0	99	±	3
Fentanyl	102	±	1	99	±	1	99	±	1	101	±	1	98	±	1	100	±	1	100	±	2
Fentanyl-d_5	101	±	0	98	±	0	97	±	3	99	±	1	96	±	1	99	±	1	99	±	1
Norfentanyl	99	±	1	98	±	1	97	±	0	99	±	2	97	±	0	96	±	1	99	±	1
Propoxyphene	100	±	0	97	±	1	97	±	2	98	±	0	94	±	0	99	±	1	101	±	1
Propoxyphene-d_{11}	102	±	0	99	±	0	97	±	3	101	±	3	97	±	1	98	±	1	100	±	2
Norpropoxyphene	95	±	2	96	±	1	99	±	4	100	±	1	94	±	2	98	±	3	98	±	4
Norpropoxyphene-d_5	96	±	1	98	±	4	99	±	0	100	±	2	97	±	3	97	±	2	100	±	1
Tramadol	99	±	3	99	±	1	99	±	1	102	±	1	99	±	0	97	±	1	98	±	3
Nortramadol	101	±	3	98	±	1	101	±	2	100	±	3	99	±	1	102	±	3	102	±	2
Benzodiazepines																						
Temazepam	101	±	1	97	±	1	100	±	4	98	±	1	97	±	1	99	±	3	97	±	4
Temazepam-d_5	96	±	9	97	±	4	100	±	1	103	±	3	95	±	2	101	±	2	101	±	2
Diazepam	98	±	1	100	±	1	102	±	2	100	±	1	100	±	0	102	±	1	98	±	1
Diazepam-d_5	99	±	2	100	±	4	101	±	0	101	±	3	100	±	4	101	±	1	96	±	2
Nordiazepam	96	±	2	96	±	0	96	±	3	96	±	2	90	±	1	100	±	1	90	±	3
Nitrazepam	95	±	2	93	±	2	93	±	1	88	±	3	82	±	1	92	±	2	85	±	1
7-aminonitrazepam	101	±	1	99	±	0	99	±	1	99	±	0	95	±	1	98	±	1	95	±	1
Oxazepam	97	±	2	98	±	2	99	±	1	100	±	2	95	±	2	97	±	0	97	±	4
Oxazepam-d_5	98	±	5	97	±	2	99	±	2	100	±	1	98	±	2	98	±	4	99	±	3
Chlordiazepoxide	100	±	2	100	±	2	98	±	2	102	±	2	99	±	3	99	±	0	102	±	1
Antidepressants																						
Dosulepin	103	±	1	99	±	2	100	±	0	100	±	0	99	±	2	99	±	3	98	±	2
Amitriptyline	102	±	1	100	±	0	102	±	2	102	±	1	99	±	2	98	±	0	99	±	3
Nortriptyline	102	±	1	99	±	0	99	±	0	98	±	2	98	±	0	98	±	3	99	±	3
Fluoxetine	101	±	3	99	±	1	98	±	2	102	±	1	96	±	4	101	±	0	100	±	0
Fluoxetine-d_6	100	±	1	98	±	2	96	±	3	99	±	0	99	±	2	102	±	5	99	±	0
Norfluoxetine	99	±	3	98	±	0	100	±	2	102	±	3	99	±	4	100	±	5	100	±	1
Venlafaxine	99	±	1	98	±	1	100	±	2	100	±	3	97	±	0	101	±	1	99	±	3
Dissociative anesthetics																						
Phencyclidine	101	±	2	99	±	2	99	±	2	101	±	2	98	±	2	97	±	2	100	±	1

PCP-d_5	101	±	1	97	±	3	100	±	1	101	±	2	98	±	2	98	±	3	98	±	0
Ketamine	100	±	3	98	±	1	97	±	1	100	±	1	97	±	0	99	±	2	98	±	0
Ketamine-d_4	101	±	3	97	±	2	98	±	0	101	±	3	98	±	1	98	±	0	99	±	3
Norketamine	97	±	2	98	±	2	97	±	0	99	±	3	97	±	2	97	±	3	97	±	1
Cannabinoids																					
THC	<MDL			101	±	6	103	±	1	89	±	4	29	±	4	91	±	3	7	±	1
THC-COOH	<MDL			100	±	4	95	±	4	56	±	8	5	±	0	71	±	3	<MDL		
THC-d_3	<MDL			103	±	6	104	±	1	91	±	4	30	±	4	91	±	4	7	±	2
Other																					
Methaqualone	97	±	1	96	±	1	96	±	2	96	±	1	92	±	3	97	±	3	92	±	1
Methaqualone-d_7	96	±	0	97	±	0	98	±	3	102	±	0	95	±	1	99	±	0	98	±	1
Sildenafil	101	±	0	100	±	1	101	±	0	102	±	2	95	±	1	100	±	1	96	±	3
Pheacetin-ethoxy-1-^{13}C	98	±	4	96	±	1	98	±	0	97	±	3	95	±	1	98	±	3	98	±	1
Drug precursors																					
Ephedrine	99	±	1	100	±	1	99	±	1	100	±	0	97	±	0	99	±	1	100	±	1
Norephedrine	99	±	2	100	±	0	99	±	0	99	±	1	95	±	3	98	±	0	100	±	0

Table S6 – Recovery of analytes after filtration through glass fibre filters

Compound	Microfibre filter recovery (%) (n=3)		
	GF/D 2.7 µm	GF/C 1.2 µm	GF/F 0.7 µm
Stimulants			
Cocaine	101 ± 2	100 ± 4	98 ± 1
Cocaine-d_3	-	100 ± 4	98 ± 1
Benzoylecgonine	101 ± 1	96 ± 3	94 ± 1
Benzoylecgonine-d_8	95 ± 1	98 ± 3	96 ± 1
Ecgonine methyl ester	107 ± 4	103 ± 1	110 ± 7
Ecgonine methyl ester-d_3	111 ± 10	101 ± 2	101 ± 1
Norbenzoylecgonine	103 ± 2	97 ± 3	97 ± 1
Norcocaine	100 ± 2	106 ± 4	100 ± 2
Cocaethylene	102 ± 1	109 ± 3	104 ± 2
Cocaethylene-d_8	100 ± 3	104 ± 3	103 ± 1
Anhydroecgonine methyl ester	99 ± 2	103 ± 4	104 ± 3
Ecgonidine	100 ± 1	105 ± 1	101 ± 1
Amphetamine	110 ± 3	97 ± 4	99 ± 3
Amphetamine-d_{11}	110 ± 6	99 ± 3	99 ± 3
Methamphetamine	108 ± 1	101 ± 4	99 ± 3
Methamphetamine-d_{14}	103 ± 3	104 ± 4	100 ± 2
Methcathinone	105 ± 7	104 ± 5	99 ± 5
BZP	98 ± 2	82 ± 16	75 ± 14
TFMPP	105 ± 5	106 ± 4	101 ± 4
Hallucinogens			
MDA	104 ± 1	102 ± 4	101 ± 2
MDA-d_5	105 ± 4	101 ± 2	101 ± 3
MDMA	98 ± 2	102 ± 2	101 ± 3
MDMA-d_5	103 ± 1	102 ± 2	100 ± 2
MDEA	98 ± 1	103 ± 5	101 ± 3
MBDB	102 ± 3	105 ± 6	104 ± 3
MBDB-d_5	103 ± 2	103 ± 4	100 ± 3
BDB	108 ± 3	105 ± 5	102 ± 2
Mescaline	105 ± 2	103 ± 2	96 ± 2
Mescaline-d_9	102 ± 2	107 ± 1	96 ± 1
LSD	101 ± 3	109 ± 10	98 ± 5
LSD-d_3	97 ± 4	109 ± 11	112 ± 5
O-H-LSD	105 ± 1	102 ± 3	99 ± 1
Human indicators			
Caffeine	103 ± 5	100 ± 1	99 ± 1
Caffeine-d_9	103 ± 0	98 ± 1	98 ± 1
1,7-dimethylxanthine	106 ± 2	97 ± 4	94 ± 1
Nicotine	82 ± 13	107 ± 18	24 ± 16
Nicotine-d_4	70 ± 17	96 ± 15	21 ± 14
Continine	101 ± 1	104 ± 4	104 ± 5
Creatinine	100 ± 4	96 ± 0	104 ± 4
Opioids and morphine derivatives			
Heroin	104 ± 6	100 ± 6	99 ± 12
Heroin-d_9	91 ± 12	83 ± 16	80 ± 18
6-acetylmorphine	100 ± 2	106 ± 3	98 ± 0
Codeine	104 ± 1	97 ± 3	98 ± 1
Codeine-d_6	-	97 ± 3	98 ± 2
Norcodeine	108 ± 2	95 ± 5	99 ± 2
Oxycodone	98 ± 6	104 ± 5	100 ± 3

Oxycodone-d_6	100	±	6	102	±	2	101	±	0
Oxymorphone	100	±	2	104	±	4	100	±	2
Morphine	99	±	3	97	±	2	105	±	2
Morphine-d_6	101	±	3	100	±	3	104	±	3
Normorphine	96	±	4	103	±	3	105	±	3
Dihydrocodeine	102	±	2	95	±	2	98	±	1
Dihydrocodeine-d_6	104	±	2	93	±	4	97	±	2
Buprenorphine	103	±	5	104	±	1	110	±	5
Buprenorphine-d_4	100	±	3	109	±	1	107	±	4
Norbuprenorphine	105	±	5	103	±	0	99	±	3
Methadone	106	±	4	109	±	2	103	±	8
Methadone-d_9	106	±	4	113	±	7	106	±	6
EDDP	96	±	6	95	±	8	99	±	3
EDDP-d_3	99	±	3	99	±	6	100	±	4
EMDP	102	±	3	97	±	2	101	±	5
Fentanyl	103	±	3	96	±	2	95	±	2
Fentanyl-d_5	101	±	4	102	±	5	120	±	6
Norfentanyl	104	±	7	106	±	0	103	±	5
Propoxyphene	109	±	6	95	±	4	105	±	5
Propoxyphene-d_{11}	104	±	5	104	±	0	108	±	5
Norpropoxyphene	106	±	2	106	±	3	107	±	7
Norpropoxyphene-d_5	100	±	4	94	±	5	95	±	8
Tramadol	98	±	2	98	±	2	96	±	1
Nortramadol	102	±	2	100	±	5	103	±	1
Benzodiazepines									
Temazepam	105	±	3	96	±	4	104	±	2
Temazepam-d_5	101	±	4	109	±	7	104	±	4
Diazepam	101	±	2	107	±	5	104	±	2
Diazepam-d_5	101	±	6	107	±	4	103	±	3
Nordiazepam	99	±	5	104	±	6	96	±	3
Nitrazepam	96	±	2	96	±	11	96	±	2
7-aminonitrazepam	91	±	2	94	±	6	95	±	2
Oxazepam	98	±	7	104	±	3	103	±	1
Oxazepam-d_5	97	±	7	127	±	5	101	±	1
Chlordiazepoxide	101	±	2	114	±	3	110	±	4
Antidepressants									
Dosulepin	99	±	4	105	±	7	95	±	8
Amitriptyline	100	±	2	104	±	6	91	±	6
Nortriptyline	104	±	5	104	±	6	93	±	6
Fluoxetine	100	±	3	108	±	10	96	±	6
Fluoxetine-d_6	94	±	7	79	±	12	97	±	10
Norfluoxetine	99	±	5	105	±	7	96	±	15
Venlafaxine	101	±	1	109	±	2	103	±	2
Dissociative anesthetics									
Phencyclidine	108	±	5	111	±	7	106	±	5
PCP-d_5	104	±	5	109	±	7	104	±	4

Ketamine	97	±	3	102	±	2	98	±	2
Ketamine-d_4	96	±	3	104	±	2	103	±	1
Norketamine	100	±	6	91	±	9	100	±	2
Other									
Methaqualone	101	±	2	114	±	6	106	±	2
Methaqualone-d_7	104	±	1	109	±	4	103	±	1
Sildenafil	113	±	7	101	±	1	84	±	12
Pheacetin-ethoxy-1-^{13}C	104	±	1	103	±	4	103	±	6
Drug precursors									
Ephedrine	104	±	3	105	±	3	102	±	2
Norephedrine	107	±	1	97	±	2	103	±	1